Canada

Fisheries and Oceans | Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Fisheries and Oceans | Pêches et Océans

Communications Directorate | Direction générale des communications

Information and Publications Branch | Direction de l'information et des publications

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial Policy

The *Journal* publishes original research articles and notes, critical reviews, perspectives (essays of opinion or hypothesis), and comments. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmation state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

A charge is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Guides

Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

Subscriptions

The *Journal* is issued monthly to form annual volumes of twelve numbers plus Supplements. Orders and inquiries regarding subscriptions must be sent directly to Supply and Services Canada, Ottawa, Ontario, Canada K1A 0S9. Prepayment is required in Canadian funds to the order of "The Receiver General for Canada."

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

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Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–1925, *Contributions to Canadian Biology and Fisheries* 1926–1934, *Journal of the Biological Board of Canada* 1934–1937 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–1979.

Politique de rédaction

Le *Journal* publie des articles et des notes fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (perspectives) et des commentaires. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influent sur les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, b) des arbitres possibles et c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Les manuscrits acceptés sont sujets à des frais de publication. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés. On trouvera dans une récente livraison du *Journal* les taux par page en vigueur.

Guides

Le guide des auteurs et celui des secrétaires d'auteurs paraissent dans la première livraison de chaque volume et sont offerts gratuitement par le *Journal*.

Abonnement

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros, et offre en plus jusqu'à deux suppléments par année. On doit faire parvenir les commandes et demandes de renseignements concernant les abonnements à Approvisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9. Les paiements doivent être faits à l'avance en devises canadiennes, à l'ordre du Receveur général du Canada.

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

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Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, Oceanic Abstracts, and Science Citation Index

Guide for Authors

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together. The paper should not be submitted or accepted for publication elsewhere.

The *Journal* considers the following types of contributions:

Articles: Studies of broad scope that are original contributions to science.

Notes: Reports of significant new knowledge arising from problems with narrow limits or important findings that warrant immediate publication before broader studies are complete. Notes should occupy no more than 3 printed (9 manuscript) pages.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science of concern to the professional and layman. References are desirable; informal style is welcome.

Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

Manuscript Submission

Submit manuscripts in triplicate to the Editor, accompanied by a covering letter that (1) states the main points and significance of the work, (2) accepts the mandatory $44 page charge, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. Original typescripts and figures (except halftones) are best retained by the author until requested.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

Editorial Practices

The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (4th ed. 1980), Spec. Publ. No. 12, American Fisheries Society. All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Fifty reprints are supplied free. Additional reprints may be puchased at the time of publication (an order form is sent with the proofs).

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 21.5 × 28 cm (8.5 × 11 in.), on only one side of the page. If possible, use paper with lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units of measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as g C/m^2/d; use g C·m^{-2}·d^{-1}.

8) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when galley proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints of figures, or originals if they do not exceed 21.5 × 28 cm, on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as ". . . is discussed" or ". . . was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting

and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents.

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . ." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the test what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit within either a column or page of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near hear."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions or the original artwork (no larger than 21.5 × 28 cm) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures should be avoided if possible, as they usually reproduce poorly. Dot-matrix® printers cannot produce sufficiently dark, solid lines to ensure legibility. Figures drafted in black india ink are always preferable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and

formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetre per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	*M*
mole	mol
mole per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. comm."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)." Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

PETERMAN, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

GORDON, D. C. JR., AND A. S. HOURSTON [ED.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LEBLOND, P. H., AND L. A. MYSAK. 1978. Waves in the ocean. Elsevier, New York, NY 602 p.

Part of book

HEALEY, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

KUTTY, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, Univ. Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

SMITH, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sous-jacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Notes : communications brèves signalant une connaissance nouvelle et significative découlant de problèmes étroitement limités et facilement définis, ou d'importantes découvertes qui méritent d'être publiées avant que des études plus vastes soient terminées. Les notes ne doivent pas dépasser 3 pages imprimées (9 pages de manuscrit).

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata, et accompagnés d'une lettre mentionnant : 1) les principaux points et l'importance du travail, 2) l'acceptation des frais de publication obligatoires de 44 $ par page, 3) des suggestions quant aux arbitres éventuels et 4) une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (4e éd. 1980), Spec. Publ. No. 12, American Fisheries Society. Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, n° 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Cinquante tirés à part sont fournis gratuitement. Des tirés à part supplémentaires peuvent être achetés au moment de la publication (un bon de commande à cet effet accompagne les épreuves).

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 21,5 sur 28 cm (8,5 sur 11 pouces), sur un côté de

la page. Si possible, employez un papier avec lignes numérotées en marge. Laissez toujours une marge d'au moins 2,5 cm.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun endroit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles ? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constatations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, date, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux et le texte, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que g C/m²/d ; utilisez plutôt g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Department d'économie, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al.*

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies des figures, ou encore les originaux, s'ils ne dépassent pas 21,5 sur 38 cm. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le

défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à . . . » ou « Recherches sur . . . ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que « . . . on y analyse » ou « . . . a été trouvé » ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail.

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer . . . nous . . . » Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possible, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendes devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à une ou deux colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit « Tableau — près d'ici ».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies ou les dessins originaux (pourvu qu'ils ne dépassent pas 21,5 sur 28 cm) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1 1/2 ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig. — près d'ici ».

On devra autant que possible éviter les illustrations préparées par ordinateur, qui ne se reproduisent pas très bien. Les imprimantes par points ne peuvent produire de lignes solides suffisamment foncées pour être lisibles. On obtiendra toujours de meilleurs résultats en utilisant de l'encre de Chine noire. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètre par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
électronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	*M*
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : « Brown (1983) compare . . . » ou « . . . on a comparé (Brown 1983) ». Si la

référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « *et al.* » : « Brown *et al.* (1983) comparent . . . » ou « . . . on a comparé (Brown *et al.* 1983) ».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « comm. pers. »

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-les dans la liste des références, avec la mention « (Sous presse) ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'aprés les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D. ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieu subarctique. Can. J. Fish. Aquat. Sci. 40: 1278–1292.

Numéro complet du journal

GORDON, D. C. JR. ET A. S. HOURSTON [ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40 (Suppl. 1): 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans, Service hydrographique du Canada, Ottawa (Ont.) 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1972. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.]. La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

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Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles « h », « min » et « s » ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12:30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, « cold water » (n.) « cold-water » (adj.); « hard water » (n.), « hardwater » (adj.), etc. L'auteur préparant une texte en anglais est prié de consulter la version anglaise du Guide.

Genetic Population Structure of Atlantic Halibut, *Hippoglossus hippoglossus*

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Atlantic halibut, *Hippoglossus hippoglossus*, have been shown to home to the same spawning site in successive years, suggesting that distinct breeding populations could exist. The species' population structure has, therefore, been studied using biochemical genetic methods to reveal possible genetic differences among fish from different spawning sites. Genetic screening of 43 loci in Faroese halibut revealed only four polymorphic loci, including one highly polymorphic fumarase locus not previously detected in halibut. Comparing these four loci among stocks from the Faroes, the Norwegian coast, and Greenland gave evidence of general genetic homogeneity over the sampling range. This lack of genetic differentiation is not taken as proof, but as strong evidence of substantial gene flow between geographical areas.

On a observé que le flétan de l'Atlantique, *Hyppoglossus hyppoglossus*, fréquente la même aire de frai pendant plusieurs années consécutives, ce qui pourrait indiquer l'existence de populations de reproducteurs distinctes. La structure des populations de l'espèce a donc été étudiée à l'aide de méthodes biochimiques adaptées à la génétique afin de mettre en évidence des différences génétiques possibles entre les poissons des différentes aires de frai. Le tri génétique de 43 loci chez le flétan dans la région des îles Faroe n'a révélé que quatre loci polymorphes, notamment un locus de la fumarase hautement polymorphe, qui n'avait pas encore été décelé chez le flétan. La comparaison des stocks des îles Faroe, de la côte de la Norvège et du Groenland pour ces quatre loci a révélé une homogénéité génétique générale à l'intérieur de l'aire d'échantillonnage. Ce manque de différentiation génétique n'est pas considéré comme une preuve, mais comme une indication très nette de l'existence d'un flux génétique important entre les régions géographiques.

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The Atlantic halibut, *Hippoglossus hippoglossus*, is an arctoboreal species, distributed across parts of the Arctic Ocean and the northern part of the Atlantic Ocean (Andriyashev 1954). Results from tagging experiments on the species indicated homing to the same spawning site in several successive years (Devold 1938, 1943; Godø and Haug 1987) and encouraged biochemical genetic studies to determine whether this apparent geographical isolation of spawners has led to genetic differentiation of the species in the North Atlantic (Mork and Haug 1983; Haug and Fevolden 1986).

Mork and Haug (1983) and Grant et al. (1984) screened a total of 43 loci, of which 7 proved to be polymorphic, in Atlantic halibut. Four of these polymorphic loci, *Ada*, *Pgi-2*, *Pgm-2*, and *Utp* (an unidentified skeletal muscle protein), had variant allele frequencies greater than 0.05 and were later used by Haug and Fevolden (1986) to investigate potential genetic effects of small- and large-scale geographical separation of halibut. Although little genetic variation was found among halibut from the areas investigated (three spawning sites in Norway and one site in western Greenland), some heterogeneity was detected which gave evidence of variation along a north–south axis (Haug and Fevolden 1986). These observations called for resampling in more southerly located halibut spawning areas.

With the recent discovery of halibut spawning grounds at 800- to 1000-m depths along the Faroe ridge southwest of the Faroes (cf. Jákupsstovu 1986), the opportunity to sample halibut from more southerly areas arose. This paper presents a comparison of the biochemical genetics of halibut from Faroese waters with results already reported from the Norwegian coast and Greenland. In addition to the four polymorphic loci reported by Haug and Fevolden (1986), a series of other loci was screened, including some not previously reported in halibut. The general amount of genetic variation in Atlantic halibut throughout its range is discussed.

Material and Methods

Samples

The material from the Norwegian coast and Greenland, which includes white skeletal muscle samples taken during the

[1]Authorship equal.

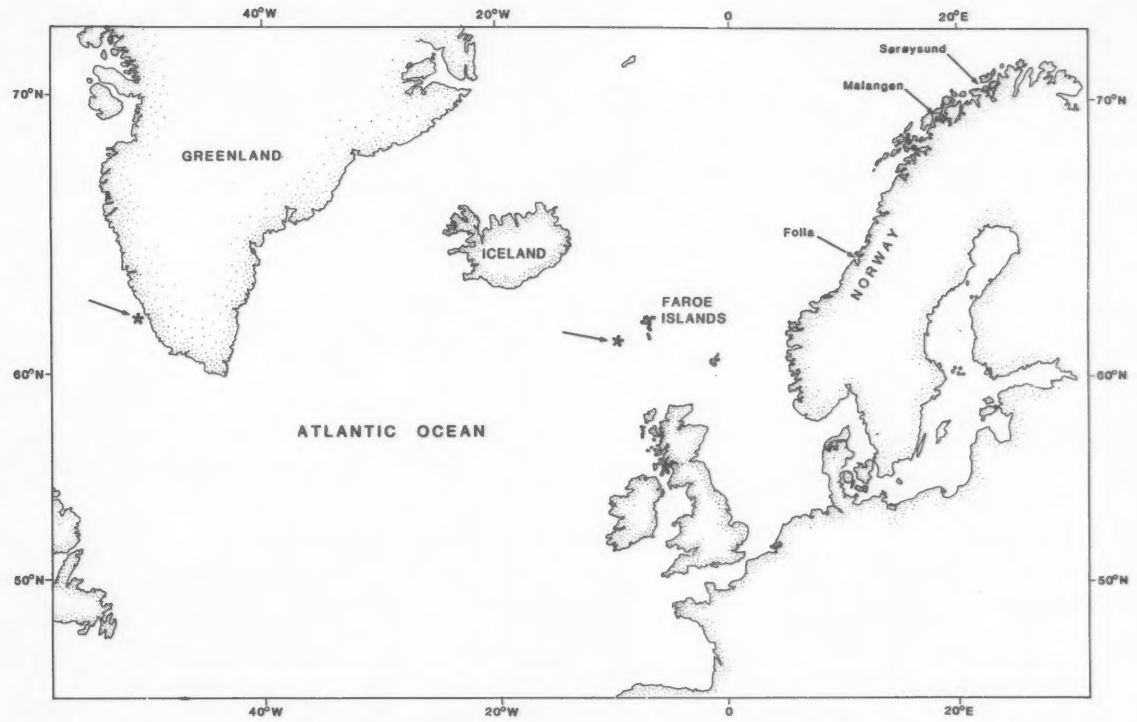

FIG. 1. Locations of sampling sites on the coast of Norway, off the Faroes, and off western Greenland (the two latter denoted with an asterisk) of the electrophoretic survey of Atlantic halibut, *Hippoglossus hippoglossus*.

spawning season from 136, 105, 41, and 53 adult fish from Sørøysund, Malangen, Folla, and Greenland, respectively (Fig. 1), was more thoroughly described by Haug and Fevolden (1986). The material from the Faroes includes 257 adult fish obtained during exploratory trawling performed with R/V *Magnus Heinason* during February 1986 (which is the halibut spawning time in this area; see Jákupsstovu and Haug 1987) in a deepwater (800–1000 m) halibut spawning area southwest of the Faroe Islands, approximate position 61°–61°40′N, 9°–10°W (Fig. 1). The white skeletal muscle tissues sampled were all taken from fresh fish which were sexed and measured for total length and frozen at −20°C. The muscle samples were shipped to Norway on dry ice and thereafter kept at −80°C until thawed for electrophoretic analysis.

Electrophoretic Methods

Twenty-three protein systems were assayed in the Faroese halibut (Table 1). *Utp* (unidentified tissue protein) was investigated using isoelectric focusing in polyacrylamide gel as described by Mork and Haug (1983). The remaining systems were assayed using common starch gel electrophoresis (Ayala et al. 1973). Three buffer systems were used: b and c as used by Haug and Fevolden (1986) and RW: gel buffer, 0.03 *M* Tris – 0.005 *M* citric acid, pH 8.5; electrode buffer, 0.06 *M* lithium hydroxide – 0.3 *M* boric acid, pH 8.1 (Ridgway et al. 1970). The staining procedures followed Fevolden and Ayala (1981) and Fevolden (1982) with modifications for *Pgi* and *Pgm* as in Haug and Fevolden (1986). Common and generally accepted criteria for scoring gene–protein systems were followed (Ayala et al. 1972, 1973). In the starch gel electrophoresis the commonest allele is assigned the number 100, while the other alleles are designated by numbers lower and higher than 100 indicating their mobility in absolute distance (millimetres) from the 100 allele. The *Utp* alleles are designated by their pIs.

Statistics

Conformity to Hardy–Weinberg equilibrium was tested using common chi-square statistics for goodness of fit. Contingency chi-square table analyses and *G*-statistics (Sokal and Rohlf 1981, box 17.4) were used to test for allele frequency heterogeneity at loci common between this study and Haug and Fevolden (1986). Rare alleles were pooled with neighbouring alleles to avoid statistical problems associated with the calculation of chi-square and *G* values using low expected allele classes. *F*-statistics (Wright 1951, 1965) were used to test the relative degree of intra- and intersample gene diversity. The basic *F*-statistics used are F_{ST}, F_{IS}, and F_{IT}. In a subdivided population, F_{ST} is a measure of allele frequency variation among subdivisions and F_{IS} and F_{IT} are measures of deviation from Hardy–Weinberg for an individual subdivision and for the compound (total population, respectively. The three *F*-statistics are related: $\bar{F}_{IS} = (F_{IT} - F_{ST})/(1 - F_{ST})$ where $\bar{F}_{IS}$ is the arithmetic mean of F_{IS} over the subdivisions. Genetic identity (Nei 1978), a non-statistical parameter, was used for paired comparison of geographical sites and to construct a dendrogram using the unweighted pair group method of arithmetic averages (UPGMA, cf. Sneath and Sokal 1973). The statistical processing of the data was facilitated by using BIOSYS-1, a population

TABLE 1. Proteins assayed and buffer systems used in the study of Atlantic halibut from the Faroe Islands. N = number of fish examined; m = monomorphic locus; p = polymorphic locus. See text for explanation of buffer systems.

Protein	E.C. No.	N	Buffer system	Locus	m/p
Acid phosphatase	3.1.3.2	134	RW	*Acph–1*[a]	m
				Acph-2[a]	m
Adenylate kinase	2.7.4.3	96	RW	*Adk*	m
Aldehyde oxidase	1.2.3.1	96	b	*Ao*[a]	m?
Aldolase	4.1.2.13	48	b	*Ald-1*[a]	m?
				Ald-2[a]	m?
Alkaline phosphatase	1.2.3.4	134	RW, b	*Aph-1*[a]	m
				Aph-3	m
Esterase	3.1.1.1	204	RW	*Est-1*	m
				Est-2	m
				Est-3[a]	m?
				Est-4[a]	m
Fumarase	4.2.1.2	257	RW	*Fum*[a]	p
Glutamate oxaloacetate transaminase (= aspartate aminotransferase)	2.6.1.1	206	RW, c	*Got-1*	m?
				Got-2	m
α-Glycerophosphate dehydrogenase	1.1.1.8	86	b	*α-Gly-1*	m
				α-Gly-2	m?
Hexokinase	2.7.1.1	86	b	*Hk*[a]	m
Isocitrate dehydrogenase	1.1.1.42	144	b	*Idh-1*	m
				Idh-2	m
Lactate dehydrogenase	1.1.1.27	144	RW, b	*Ldh*	m
Leucine aminopeptidase	3.4.11.1	134	RW	*Lap-1*	m
				Lap-3	m
Malate dehydrogenase	1.1.1.37	184	RW	*Mdh-1*	m
				Mdh-2	m
Malic enzyme	1.1.1.40	48	RW	*Me-1*	m?
				Me-2	m?
Mannosephosphate isomerase	5.3.1.8	96	RW	*Mpi-1*	m?
				Mpi-2	m?
Octanol dehydrogenase	1.1.1.73	96	c	*Odh-1*[a]	m
				Odh-2[a]	m
Phosphoglucose isomerase (glucosephosphate isomerase)	5.3.1.9	257	RW, b	*Pgi-1*	m
				Pgi-2	p
Phosphoglucomutase	2.7.5.3	257	RW	*Pgm-1*	m
				Pgm-2	p
Sorbitol dehydrogenase	1.1.1.14	48	b	*Sdh*[a]	m?
Xanthine dehydrogenase	1.2.1.37	96	b	*Xdh*[a]	m
General protein		206	RW	*Prot-1*[a]	m
				Prot-2[a]	m
				Prot-3[a]	m?
				Prot-4[a]	m?
				Prot-5[a]	m
Unidentified tissue protein[b]		74		*Utp*	p

[a]Not previously assayed in Atlantic halibut.
[b]Analyzed by isoelectric focussing.

genetics computer program developed by Swofford and Selander (1981).

Results

In the Faroese sample, 43 loci were assayed (Table 1). The resolution of 13 of these was insufficient to confirm the impression of monomorphism. Of the remaining 30, all but four were monomorphic; that is, the frequency of the most common allele was greater than 0.99. The four polymorphic loci coded the enzymes fumarase (FUM), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), and an unidentified skeletal muscle protein (UTP). Adenosine deaminase (ADA), which was proved polymorphic by Haug and Fevolden (1986), was also run in the Faroese sample, but the activity was too low for it to be reliably scored.

PGI and PGM were coded at loci with banding characteristics that corresponded with those of Atlantic halibut reported by Grant et al. (1984) and Haug and Fevolden (1986). The faster of two loci at each system was polymorphic. A description of the banding patterns of the polymorphic *Utp* locus was given by Mork and Haug (1983). The fourth polymorphic locus, *Fum*, appeared with one zone of anodally migrating bands. The tetrameric enzyme (Harris and Hopkinson 1976) had satellite bands and was somewhat blurred at times, but heterozygotes and homozygotes could easily be distinguished (Fig. 2). Because of the discovery of this new highly polymorphic locus in Atlantic halibut, we also analyzed the four samples of halibut from the

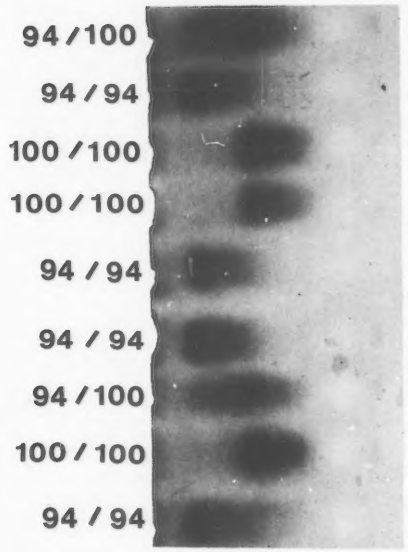

FIG. 2. Starch gel zymogram of fumarase (*Fum*) variants in Atlantic halibut, *Hippoglossus hippoglossus*.

Norwegian coast and Greenland (Haug and Fevolden 1986) for the *Fum* locus.

For comparison, in Table 2 the allelic frequencies and frequencies of heterozygotes at the polymorphic loci *Pgi-2*, *Pgm-2*, and *Utp* for the three Norwegian coast samples and the Greenland sample originally presented by Haug and Fevolden (1986) are given along with the new Faroese data and the results from the analysis of the *Fum* locus in the Norwegian coast and Greenland material.

The frequency of heterozygotes at the four polymorphic loci (Table 2) plus the 26 definitely monomorphic loci (Table 1) gives an average expected frequency of heterozygotes for Faroese halibut of 0.049 (±0.025 SE).

The four polymorphic loci in the Faroese sample gave evidence of a population in Hardy–Weinberg equilibrium; that is, the frequency of genotypes was as expected for a randomly mating population ($p > 0.247$). No significant deficits or surpluses of heterozygotes at the *Fum* locus were found in the Norwegian coast samples or in the sample from Greenland ($p > 0.241$, Table 2).

The skewed sex ratio in the Faroese sample, with 2.4 males per female, was not accompanied by allele frequency differences between the two sexes. No loci showed intersex heterogeneity in the Faroese sample ($p > 0.05$).

Gene diversity analyses using *F*-statistics (Wright 1951, 1965) gave further evidence of homogeneity over the sampling range (Tables 3 and 4). The significance of F_{IS} has been tested by the formula of Li (1955): $\chi^2 = N \cdot F^2_{IS}$ where N is the sample size. None of the F_{IS} values were significant according to this formula (Table 3). The summary of *F*-statistics (Table 4) shows that the F_{IT} values are only slightly higher than the F_{IS} values; that is, the compound population is no more in Hardy–Weinberg disequilibrium than are the individual populations. None of the F_{IT} values were significant at the 0.05 level using the significance method of Brown (1970) ($|F_{IT}| \cdot \sqrt{N}$ should be greater than 1.96 for significance at the 5% level; N is the sample size).

The generally low values of F_{ST} (Table 4) show that the gene diversity caused by variation among samples, or subdivisions, is small. It varies between 0.1% for *Fum* and 2.7% for *Pgm-2*. On average, about 1% of the gene diversity is found between samples, while 99% of the diversity is allocated within samples.

Analyses of heterogeneity among samples (both χ^2 tests and *G*-tests, Table 5) reveal homogeneity at three loci, while *Pgm-2* shows significant heterogeneity among the five subdivisions, in accordance with the higher value of F_{ST} for *Pgm-2* than for the other polymorphic loci (Table 4). The heterogeneity at *Pgm-2* (Table 5) is still significant at the 5% level after mod-

TABLE 2. Allelic frequencies and frequencies of heterozygotes (*H*, observed/expected) at the gene loci *Fum*, *Pgi-2*, *Pgm-2*, and *Utp* of Atlantic halibut from various sites in the North Atlantic. N = number of fish analyzed.

		Norwegian coast[a]				
Locus	Alleles	Folla	Malangen	Sørøysund	Greenland[a]	Faroes
Fum	*N*	39	89	125	51	246
	94	0.462	0.444	0.488	0.451	0.449
	100	0.538	0.556	0.512	0.549	0.551
	H	0.410/0.503	0.438/0.496	0.464/0.502	0.510/0.500	0.459/0.496
Pgi-2	N	38	94	136	53	257
	94	0.039	0.101	0.077	0.132	0.088
	100	0.961	0.899	0.919	0.868	0.907
	102	—	—	0.004	—	0.006
	H	0.079/0.077	0.169/0.183	0.162/0.150	0.226/0.231	0.171/0.171
Pgm-2	*N*	38	95	136	53	254
	96	0.118	0.279	0.239	0.245	0.331
	98	—	0.005	0.015	0.019	0.004
	100	0.882	0.716	0.739	0.736	0.663
	102	—	—	0.007	—	0.002
	H	0.184/0.212	0.358/0.412	0.412/0.398	0.434/0.318	0.425/0.451
Utp	*N*	41	105	134	51	74
	6.9	0.220	0.152	0.172	0.196	0.223
	7.0	0.780	0.848	0.828	0.804	0.777
	H	0.341/0.347	0.241/0.260	0.254/0.285	0.235/0.318	0.365/0.349

[a]The *Pgi-2*, *Pgm-2*, and *Utp* results from the Norwegian coast and Greenland are taken from Haug and Fevolden (1986).

TABLE 3. $\bar{F}_{IS}$ values (Wright 1951, 1965; means over alleles) for the five sampling sites of Atlantic halibut. None of the $\bar{F}_{IS}$ values were significant at the 5% level using the significance test of Li (1955) (see text).

Locus	Sampling site				
	Faroe Is	Folla	Malangen	Sørøysund	Greenland
Fum	0.072	0.175	0.112	0.071	−0.030
Pgi-2	−0.005	−0.041	0.122	−0.084	0.012
Pgm-2	0.056	0.118	0.127	−0.038	−0.090
Utp	−0.053	0.003	0.041	0.108	0.254

TABLE 4. Summary of *F*-statistics (Wright 1951, 1965) at the four polymorphic loci over the five sampling sites of Atlantic halibut. None of the F_{IT} values were significant at the 5% level using the significance test of Brown (1970) (see text for explanation).

Locus	$\bar{F}_{IS}$	F_{IT}	F_{ST}
Fum	0.080	0.081	0.001
Pgi-2	0.011	0.022	0.011
Pgm-2	0.027	0.053	0.027
Utp	0.067	0.072	0.005
Mean	0.054	0.064	0.010

TABLE 5. Chi-square test and *G*-test of heterogeneity at four polymorphic loci among Atlantic halibut from five sampling sites. *0.01 < p 0.05; **0.001 < p < 0.01; ***p < 0.001; ns = not significant.

Locus	χ^2	G	df
Fum	1.228 ns	1.16 ns	4
Pgi-2	5.577 ns	5.78 ns	8
Pgm-2	17.963**	19.78***	12
Utp	3.957 ns	3.92 ns	4
Total	28.725*	30.64*	28

ifying the significance level by dividing p (0.05) by the number of loci (4) included in the heterogeneity test (*I*-error, Cooper 1968). The additive chi-square and *G* values (bottom line of Table 5) show that the *Pgm-2*-divergence is in fact sufficient to reveal significant heterogeneity over the group of loci.

Clustering samples according to their paired genetic identity values (*I*) over the four polymorphic loci reveals two main "groupings" (Fig. 3), one consisting of one sample only, Folla, and the other consisting of the remaining four samples. The branching is largely due to the variation at *Pgm-2* and *Pgi-2*. It was shown by Haug and Fevolden (1986) that the heterozygosity for the Folla sample at these two loci was less than half that of the other samples and the commonest allele was overrepresented. The present cluster is largely identical to the one presented by Haug and Fevolden (1986, fig. 2a), but the larger grouping now also includes the Faroese halibut. It should be noted that *I* values of 1.000, which appears from Fig. 3 between the Faroes and Greenland and between Malangen and Sørøysund, with the number of decimals used, do not necessarily reflect absolute identity.

Discussion

If only the 30 definitely mono- or polymorphic loci (Table 1) are included, the present estimate of average heterozygosity in Atlantic halibut is more than 10 times that of Mork and Haug (1983) (0.4% based on studies of 25 loci). However, if the 13

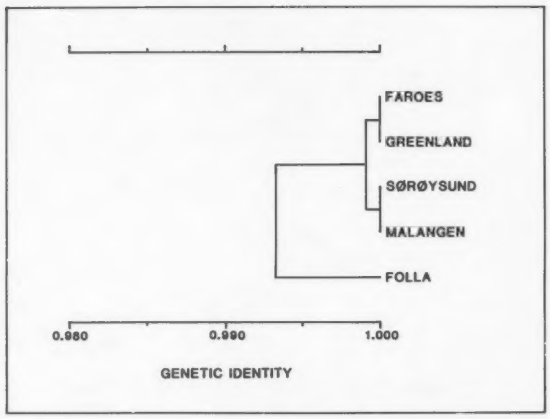

FIG. 3. Cluster dendrogram based on Nei's (1978) unbiased genetic identity coefficients for comparison of four polymorphic gene loci in Atlantic halibut, *Hippoglossus hippoglossus*, from the different sampling sites.

remaining loci in Table 1 are confirmed to be monomorphic (examination of other tissues may give better resolution of these loci), the expected heterozygosity over 43 loci is lowered to 0.033 (±0.017). An average heterozygosity of Atlantic halibut of 0.024 (±0.014) is estimated from the 35 loci studied by Grant et al. (1984). These low values of *H* and the low number of polymorphic loci indicate that halibut heterozygosity is modest compared with the mean of 5% estimated for teleosts in general (Nevo 1978).

Our initial hypothesis that Atlantic halibut show genetic heterogeneity along a north–south axis (Haug and Fevolden 1986) has not been supported by the present study. On the contrary, samples from the southernmost site sampled so far, the waters around the Faroe Islands, were clustered together with the northernmost samples from sites in northern Norway and with the Greenland sample (Fig. 1). Thus, the only sample that still seems to stand out from the others is the Folla sample from mid-Norway (Fig. 1 and 3). Chi-square tests had revealed significant differences at the *Pgm-2* and *Pgi-2* loci when single loci differences between Folla and northern Norway and Folla and Greenland were considered, and it was suggested that Folla could represent a genetically distinct stock (Haug and Fevolden 1986). The major contribution to the present significant intersample heterogeneity at *Pgm-2* (Table 5) is also from the Folla sample. The dominance of females in the Folla sample (Mork and Haug 1983) should not account for the discrepancy, since it had been shown from the other sampling sites that no sex differences in allele frequencies existed at any locus (Haug and Fevolden 1986; this study). The conformance to Hardy–Weinberg equilibrium for the Folla sample (Haug and Fevolden 1986) gives no evidence of a Wahlund effect (a physical mixture of genetically distinct populations) at the sampling site. No attempts have been made to correlate variation in catalytic activities of PGM variants in halibut to environmental variables. It is therefore not possible at present to explain the heterogeneity by environmental selection (selective mortality).

Only 38 fish were available for analysis from the Folla spawning site. This number may be critical for satisfactory sta-

tistical precision. It cannot be totally neglected therefore, that a larger sample size from Folla may have revealed that *Pgm-2*, and perhaps also *Pgi-2*, really are diagnostic loci for detecting population isolates, although, more conservatively, it is tempting to suggest that the heterogeneity caused by the Folla sample has no biological significance.

The general impression is that the North Atlantic population of halibut is very homogeneous in terms of allele frequencies as indicated both by the genetic identity values between samples (Fig. 3) and by the small fraction of the total gene diversity which is explained by differences among samples (1%) despite the large geographical distances involved. In other words, there is no convincing evidence of genetically distinct breeding stocks from the biochemical genetics data. These observations are consistent with previous reports of several other flatfish species (Grant et al. 1983) as well as of Atlantic cod, *Gadus morhua* (Mork et al. 1985), and seem to be a common feature among marine fish which are not prevented by physical barriers from migration between localities (cf. Gyllensten 1985).

The lack of genetic differentiation in halibut is found despite the observed homing of adult halibut to the same spawning site in several successive years (Devold 1938, 1943; Godø and Haug 1987). Under a neutral model, such a low amount of genetic differentiation among stocks of fish may be explained as a result of substantial gene flow between stocks. Under an adaptive selection model the possibility of stabilizing selection must also be considered and could seem reasonable in this case, given the very uniform conditions under which halibut usually live. However, the observed lack of genetic differentiation probably reflects migrations between stocks. Tag and recapture studies of immature specimens have given evidence of extensive long-distance migration in a period of several years lasting between emigration from nursery areas and recruitment to the spawning stock, although tagged fish have as yet not been observed going from one spawning ground to another (Godø and Haug 1987).

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Contribution of Early Life Stages to Interannual Variability in Recruitment of Northern Anchovy (*Engraulis mordax*)

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Peterman, R. M., M. J. Bradford, N. C. H. Lo, and R. D. Methot. 1988. Contribution of early life stages to interannual variability in recruitment of northern anchovy (*Engraulis mordax*). Can. J. Fish. Aquat. Sci. 45: 8–16.

We tested Hjort's and Lasker's hypotheses that the abundance of recruits in fishes is determined at an early life stage. Using 13 yr of data on components of population dynamics of the well-studied northern anchovy (*Engraulis mordax*), we reconstructed the abundance of anchovy in each year at three stages: eggs, 4.5-d-old yolk-sac larvae, and 19-d-old larvae. No abundance measure was significantly correlated with age 1 recruits, resulting in rejection of Hjort's and Lasker's hypotheses. We give reasons why the low correlations are not an artifact of estimation error. The lack of correlation exists because of the large variability (CV = 171%) in survival rate between age 19 d and age 1 yr. Therefore, attempts to understand interannual variability in recruitment in this, and perhaps other, marine fish species may have to rely not only on data on eggs and larvae, but especially on data on abundances estimated after 20 d, closer to the age at recruitment.

Les hypothèses de Hjort et de Lasker, selon lesquelles le nombre de recrues chez les poissons est déterminé au cours des premiers stades de vie, ont été testées. À partir des données couvrant une période de 13 ans provenant des études sur la dynamique des populations de l'anchois du Pacifique (*Engraulis mordax*), on a retracé l'abondance annuelle des anchois à trois stades différents, soit les stades oeuf, alevin vésiculé de 4,5, et alevin de 19. On n'a observé aucune corrélation significative entre les différentes mesures d'abondance et le nombre de recrues d'âge 1, et les hypothèses de Hjort et de Lasker ont donc été rejetées. On explique pourquoi ces faibles corrélations ne résultent pas d'un artefact dû à l'erreur d'estimation. L'absence de corrélation s'explique par la grande variabilité (CV = 171 %) du taux de survie entre les âges 19 j et 1 a. Pour comprendre la variabilité interannuelle du recrutement chez l'anchois, et peut-être chez d'autres espèces de poissons marins, les données concernant les oeufs et les alevins peuvent être insuffisantes; peut-être serait-il préférable de se fier surtout aux données sur l'abondance après l'âge de 20 j, c'est-à-dire à un âge plus rapproché du moment du recrutement.

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One of the best examples of a paradigm in fish population biology is Hjort's (1913, 1926) hypothesis that the annual abundance of recruits (year-class strength) is established at an early life stage, soon after larvae absorb their yolk sac. This hypothesis was based on the observed high mortality rates of these young fish, and on their planktonic nature, which potentially makes them extremely vulnerable to starvation, predation, and transport by movement of water away from habitats favorable to subsequent growth and survival. This "critical period" hypothesis has stimulated considerable research and has formed the foundation of many attempts to explain interannual variability in recruitment (e.g. Bannister et al. 1974; Cushing 1975; Sinclair et al. 1985). May (1974) and W. C. Leggett (pers. comm.) reviewed the literature and showed that there is not strong support for Hjort's hypothesis, yet it still is the basis of considerable research (e.g. Walsh et al. 1981; Wroblewski 1984; and others).

Lasker's (1975, 1981) stable ocean hypothesis is a special case of Hjort's paradigm. Lasker proposed that wind-driven turbulent mixing of the upper ocean could cause high mortality of first-feeding fish larvae through dissipation and dilution of patches of larval food. Consistent with this is the observation that starvation is the major source of mortality for first-feeding larvae (Theilacker 1986), although this does not appear to be a direct cause of death for older larvae (O'Connell 1980). The second part of Lasker's hypothesis was that these turbulent mixing conditions would lead to a poor year-class.

Recently, Peterman and Bradford (1987) tested the turbulent mixing mechanism of Lasker's stable ocean hypothesis and found that, indeed, high mortality rates of young northern anchovy (*Engraulis mordax*) larvae (up to age 19 d) occurred in years when spawning seasons had many periods of high-speed winds. Years with few periods of high winds were asso-

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ciated with low larval mortality rates. If Hjort and Lasker are correct in hypothesizing that mortality at this early stage has a strong influence on subsequent recruitment, then we should expect a positive correlation between the number of larvae surviving through this early larval period and subsequent recruitment. We arbitrarily defined recruitment as the abundance of fish of age 1 yr.

For the northern anchovy, previous analyses (Ahlstrom 1965; Methot 1983; Smith 1981) suggest that the number of surviving larvae is *not* related to recruitment. However, no correlation statistic was calculated in these cases either because of too few years of data (Methot 1983) or because only qualitative indices of recruitment were available. Both limitations have been rectified; estimates of age 1 recruitment since 1965 are now available from stochastic virtual population analysis (VPA) (Methot 1986, 1988).

In this paper, we reconstruct the annual abundance of northern anchovy at each of several early life stages, using in most cases annual observations on components of egg production and on mortality rates of eggs and larvae. We then estimate the extent to which interannual variation in abundance at each of these early life stages contributed to interannual variation in abundance of age 1 recruits of northern anchovy from 1965 to 1985. Thus, we test Hjort's and Lasker's hyotheses for this northern anchovy population.

Methods

We used data on the central population of northern anchovy, which spawns primarily during January–April in waters off the coast of southern California. This species is one of the dominant fish in the California Current, with a mean spawning biomass of 500×10^3 metric tonnes (t) from 1965 to 1985 (Methot 1986), and it has been intensively studied since the 1950's by the California Cooperative Oceanic Fisheries Investigations (CalCOFI) (see annual volumes of CalCOFI Reports and Smith 1985).

To initiate the reconstruction of abundances of early life stages (eggs, yolk-sac larvae, and 19-d-old larvae or prerecruits), we used numbers of fish at each age from 1 to 5+ yr estimated for 1965–85 by Methot's (1986) VPA (Table 1).

Egg Production, E

Total egg production by the anchovy population in a given year, E_j, was estimated by

$$(1) \quad E_j = \sum_{i=1}^{5} 0.5 N_{ij}\, p_{ij}\, F_{ij}\, S_{ij},$$

where i = age-class, j = year, 0.5 = fraction of females (Bindman 1986), N_{ij} = number of adults by age and year, from Table 1, p_{ij} = proportion of fish mature in each age by year, F_{ij} = batch fecundity (eggs per female per spawning), and S_{ij} = spawning frequency, or number of spawnings per year per female of age i.

A constant sex ratio was used because annual estimates are unreliable due to small samples and a tendency for segregation between sexes in survey catches (Klingbeil 1978).

The proportion of females of a given age which was mature was set = 1.0 for ages ≥2 based on Parrish et al. (1986) and, for age 1 fish only, was made an increasing function of the mean January–February water temperature as measured at Scripps pier (Methot 1986). For years when the fraction of mature age 1 fish was estimated from field samples (Methot 1986), we used the mean of this estimate and the one predicted for that year from the water temperature by Methot's (1986) temperature-dependent function (Table 2). This approach reflects the imprecision of the field samples. In years without field samples, we used Methot's function and the observed temperature to estimate age 1 maturity.

TABLE 1. Millions of fish at time of spawning in each age group of the central population of northern anchovy. Data from Methot's (1986) virtual population analysis. This table only shows data for those years that were relevant to the analysis (other years had no data on early life stages).

Year	Age 1	Age 2	Age 3	Age 4	Age 5+
1965	10 794	5 744	1 965	1339	635
1966	5 494	5 695	2 917	959	924
1969	14 241	7 779	1 707	473	528
1972	56 233	26 496	5 151	1013	375
1975	17 806	21 822	17 177	6118	2547
1978	9 390	13 462	1 211	754	1099
1979	76 561	3 457	4 276	355	500
1980	58 144	24 737	885	966	170
1981	45 273	18 913	6 634	208	233
1982	15 537	11 063	3 319	908	47
1983	51 305	3 667	1 728	392	84
1984	24 174	17 641	1 046	438	107
1985	54 693	8 980	5 718	313	150

Because northern anchovy are multiple spawners with an indeterminate annual fecundity (Hunter and Macewicz 1980), we estimated the number of eggs produced per mature female per year from two components: eggs per female per spawning (batch fecundity) and number of spawnings per year per female (spawning frequency).

Batch fecundity, F

Eggs per female per spawning (F) was calculated from the batch fecundity relations reported by Hunter et al. (1985), which are linear functions of female weight:

$$(2) \quad F_{ij} = a + b(0.95 W_{ij}).$$

The weight of each female of age i in year j was multiplied by 0.95 because Hunter et al.'s (1985) batch fecundity relations used gonad-free weight which is 95% of total weight (Hunter and Macewicz 1980). Table 2 gives the annual values for the a and b parameters in equation (2); long-term mean parameter values (Hunter et al. 1985) were used in years when field estimates of batch fecundity relations were not available. Annual weights of female spawners at each age (Table 3) were obtained from California Department of Fish and Game surveys (Methot 1986). Again, long-term means were used for years when it was not possible to estimate weights from field samples.

Spawning frequency, S

Parrish et al. (1986) showed that older females produce more egg batches per year than small females. We therefore used the following relation between number of egg batches per female per year (spawning frequency, S) and weight:

$$(3) \quad S_{ij} = c + dW_{ij},$$

where $c = -6.136$, $d = 1.2878$, and W_{ij} = weight (grams). Paramaters c and d were estimated by linear regression of our "adjusted" version of Parrish et al.'s (1986) data for number

TABLE 2. Annual parameter estimates for components of egg production and mortality of eggs and larvae by the year in which spawning occurred. Maturity, p_1, is the fraction of age 1 females which were mature in year j. Fecundity parameters a and b refer to equation (2); missing parameter estimates were filled in with long-term means, denoted by an asterisk. Embryo mortality parameters α and t refer to equation (4) and larval mortality parameters β and u to equation (5). Age 1 recruits are those resulting from spawning in the year given in column 1. Data sources are given in the text.

Year	Maturity	Fecundity		Embryo mortality		Larval mortality		Age 1 recruits
(j)	(p_1)	a	b	α	t	β	u	(10^6)
1965	0.28	−1104*	583*	0.19	4.91	2.42	18.27	5 494
1966	0.57	−1104*	583*	0.42	4.91	1.84	20.35	7 911
1969	0.82	−1104*	583*	0.19	4.79	2.15	18.33	23 675
1972	0.10	−1104*	583*	0.25	4.54	1.73	18.35	68 509
1975	0.23	−1104*	583*	0.44	4.89	1.88	18.95	10 617
1978	0.99	−1104*	583*	0.59	4.24	1.66	19.47	76 561
1979	0.55	−4410	658	0.48	4.91	2.22	18.62	58 144
1980	0.98	−1891	535	0.36	3.05	1.22	18.63	45 273
1981	0.92	−1979	714	0.38	3.10	1.53	18.56	15 537
1982	0.75	−180	586	0.36	4.42	1.81	18.13	51 305
1983	0.96	−1002	559	0.46	3.82	2.05	18.88	24 174
1984	0.94	−554	505	0.36	4.26	1.47	19.74	54 693
1985	0.63	−2040	648	0.28	4.93	2.13	20.96	47 011

TABLE 3. Total weight (g) per spawning fish. Data from Methot (1986), derived from California Department of Fish and Game winter surveys. Years with missing data (denoted by an asterisk) were filled in with the long-term mean for that age.

	Age				
Year	1	2	3	4	5+
1965*	8.7	14.6	18.3	21.2	24.3
1966*	8.7	14.6	18.3	21.2	24.3
1969*	8.7	14.6	18.3	21.2	24.3
1972*	8.7	14.6	18.3	21.2	24.3
1975*	8.7	14.6	18.3	21.2	24.3
1978	10.4	15.4	19.7	22.2	22.2
1979	9.2	18.3	21.7	24.8	24.3
1980	7.1	15.0	17.9	24.8	29.5
1981	9.8	13.4	18.3	20.2	27.7
1982	9.5	16.6	18.8	21.2	22.2
1983	8.9	11.0	17.5	19.7	24.3
1984	4.8	12.4	13.8	18.8	22.2
1985	10.6	15.4	18.4	21.2	24.3

of egg batches for fish aged 1, 2, and 3 yr on our mean spawning weight at those ages ($r = 0.996$).

We "adjusted" Parrish et al.'s (1986) data as follows. These authors provided age-specific monthly spawning frequencies (number of egg batches per female per year) for each age 1–3 and age 4+ fish, averaged over many years. However, for age 1 fish, the spawning frequencies in Parrish et al. (1986) were calculated from samples which included immature fish. To obtain an estimate of spawning frequency of *mature* age 1 fish only, we divided Parrish et al.'s (1986) spawning frequency for all age 1 fish by the average fraction of age 1 fish that matured during those years. This fraction was 0.905, as estimated by the fraction of age 1 fish mature during March, the month with the highest mean proportion of spawning age 1 fish (Parrish et al. 1986). This method assumed that the fish that were immature in that month would not spawn at all that year. In addition, the monthly spawning frequencies for all ages were divided by 0.95, a correction for sampling bias noted by Parrish et al. (1986). The resulting "adjusted" estimates of spawning frequency were used to estimate the parameters of equation (3) by regression.

The number of spawnings each year for mature age 1–3 fish was the sum of the monthly spawning frequencies for each age over the 12-mo interval, December–November. November was the month with least spawning and formed a natural break in the annual spawning cycle.

We then estimated the spawning frequencies for each age in each year from equation (3), using weights of spawning females of each age in that year. The product of the components shown in equation (1) thus gave an estimate of the total number of eggs produced each year by the central population of northern anchovy.

Early Larvae, L

Lo (1985) calculated the mean instantaneous daily mortality rate of each annual cohort of eggs between time of spawning and mean age of yolk-sac larvae. We used her "Series 2" estimates of this rate, α (see Table 2). Lo also estimated the time between these two stages, t, for each year (Table 2) because it was affected by water temperature (Lo 1983). The mean age of yolk-sac larvae from data in Table 2 averaged 4.4 d from time of spawning; such larvae were 3–4 mm in length (Lo 1983). Standard CalCOFI sampling cruises for eggs and larvae were made only every third year between 1966 and 1978; thus, our analysis here is limited to the 13 yrs sampled by CalCOFI between 1965 and 1985, when both age 1 recruitment data and egg mortality data were available (see section on Recruits below). We used Lo's (1985) equation to calculate the number of eggs surviving to mean age of yolk-sac larvae (L_j):

$$(4)\quad L_j = E_j e^{-\alpha_j t_j}$$

where α_j = instantaneous daily mortality rate in year j and t_j = temperature-dependent mean age of yolk-sac larvae in year j, in days since fertilization. Annual values for the α_j and t_j parameters are given in Table 2. In this species, first feeding of larvae is initiated only after the yolk is absorbed (Theilacker and Dorsey 1980). Thus, abundance of the yolk-sac larvae, L_j from equation (4), is estimated just prior to onset of first feeding.

Prerecruit Larvae, PR

Lo (1985) also estimated for each year the mortality coefficient of larvae from the mean age of yolk-sac larvae (just before the start of first feeding) to an average of 19 d after spawning (~10 mm length). Larvae larger than this were not included in the analysis because of their increased ability to avoid standard bongo nets (Smith 1981; Hewitt and Methot 1982: Folkvord and Hunter 1986; Lo 1986). This early larval period spans yolk-sac absorption, initiation of first feeding, and the early larval stage up to 19 d of age. To simplify terminology, we refer to the latter, 19-d-old larvae as prerecruits. Lo (1985) assumed that mortality rate varied with larval age, and she estimated the duration of this period for each year. Table 2 gives her annual estimates of the β mortality coefficient and times at the beginning and ending of this period, t and u, respectively. We then calculated for each year the survival of yolk-sac larvae, L_j, to prerecruits, PR_j, with Lo's (1985) equation:

$$(5)\quad PR_j = L_j\,(u_j/t_j)^{-\beta_j}$$

where t_j = same as in equation (4) and u_j = mean age of larvae at end of the sampled period in year j, in days since fertilization.

Recruits, R

Because no field estimates exist for annual mortality rates between prerecruits (19-d-old larvae) and age 1, we obtained annual estimates of abundance of age 1 recruits, R_j, from Methot's (1986) virtual population analysis. These recruits (Table 2) represent abundance as of 15 February each year. Thus, the 1966 estimate of 5494×10^6 age 1 fish is the recruitment at age 1 resulting from spawning in early 1965. Because no reliable adult age structure data were available before 1965, our data set for age 1 recruits was restricted to 1965 and later. This excluded the pre-1965 data on earlier life stages from our analysis of the effect of these early stages on subsequent recruitment.

Life Table

To compare the extent of mortality suffered by a cohort during each life stage up to recruitment, we calculated standard survival rates (s, not to be confused with S, which is spawning frequency as defined above) and instantaneous mortality rates for each year:

$$(6)\quad \begin{aligned} s_{j1} &= L_j/E_j, \\ s_{j2} &= PR_j/L_j \\ s_{j3} &= R_j/PR_j \end{aligned}$$

where the second subscript, $k = 1$, 2, or 3, on s indicates the life phase. These fractions surviving were also converted to total instantaneous mortality rates for the respective periods:

$$(7)\quad M_{jk} = -\log_e (s_{jk}).$$

We used statistics on these 13 yearly M_{jk} to construct a life table for the central population of northern anchovy.

Analysis

We took natural logarithms of abundances at all stages to calculate correlations because recruitment of marine fishes is frequently lognormally distributed (Hennemuth et al. 1980) and because logarithms of our abundances did not deviate substantially from a normal distribution (although there were too few data points to test this formally).

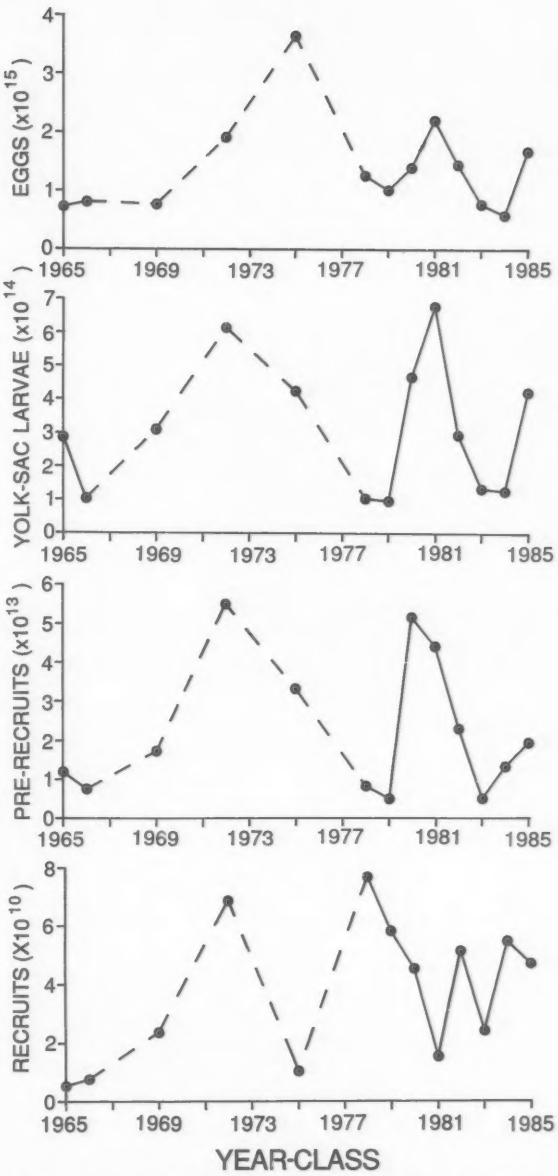

FIG. 1. Estimated abundances of early life stages for the central population of northern anchovy by year-class. Abundances of the first three stages are from this paper, and age 1 abundances are from Methot's (1986) VPA. Broken lines connect CalCOFI survey years before 1978, in which early life history variables were estimated only every third year from 1966 to 1978. Actual abundances in non-CalCOFI years could have been much higher or lower than the locations of the broken lines; dashes are provided only to aid comparison of observed points.

Results and Discussion

Recruitment Variability

Figure 1 shows estimated annual abundances of each life stage for the northern anchovy and Table 4 gives summary

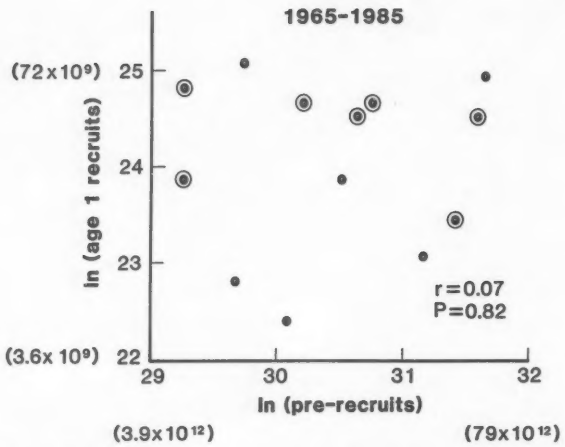

FIG. 2. Relationship between $\log_e$(prerecruit abundance, ~age 19 d) and resulting abundance of $\log_e$(age 1 recruits) for 1965–85 year-classes. Circled data points are from those years in which all components in the reconstruction of prerecruits were observed (see General Discussion section).

TABLE 4. Summary statistics of the 13 yr of abundances of northern anchovy, in numbers at each stage. Geometric mean column is the mean of the natural logs of abundances.

Stage	Arithmetic mean	Standard deviation	CV	Geometric mean
Eggs	1.40×10^{15}	8.39×10^{14}	0.599	34.74
Larvae (4.5 d)	3.12×10^{14}	1.99×10^{14}	0.638	33.16
Prerecruits (19 d)	2.26×10^{13}	1.76×10^{13}	0.780	30.44
Recruits (1 yr)	3.76×10^{10}	2.42×10^{10}	0.643	24.07

statistics.

Analysis of our 13 yearly abundances of early life stages and age 1 recruitment does not support Hjort's or Lasker's hypothesis that recruitment is established at an early stage in northern anchovy. No correlation between abundance at early life stages and recruitment was significant, including the one between age 1 recruits and prerecruits (those larvae which survived through 19 d) (Fig. 2; Table 5). Thus, even though the wind speed index of turbulent mixing explained 65% of the interannual variation in larval mortality during the "critical" first-feeding phase (Peterman and Bradford 1987), the numbers of larvae surviving through this period were not correlated with subsequent recruitment (r was only 0.07). Hjort's and Lasker's hypotheses thus are not applicable to our 13 yr of data.

Mortality between the larval stage at 19 d old and age 1 is sufficiently variable among years to destroy the correlations between abundance at all early life stages and recruitment. However, variable mortality *prior to* 19 d is not the cause of these low correlations. Lo's (1985) estimates of mortality rates and time at stage varied among years but still led to significant correlations between egg abundance and resulting yolk-sac larvae and between these larvae and subsequent prerecruits (Table 6). Yet neither the correlation between eggs and age 1 recruits nor the correlation between yolk-sac larvae and age 1 was significant (Table 5). Based on instantaneous mortality rates (Table 7), mean survival through the stage from prerecruits to age 1 was lowest of the three stages (Table 8); only 0.34% of the 19-d-old larvae survived to age 1 recruits, on average. Most important, however, this stage was the most variable, having a coefficient of variation (CV) in survival rate of 1.71, almost three times that of the egg-to-larval stage (Table 8). Thus, the interannual variability in mortality between successive early life stages (egg-to-larvae, larvae-to-prerecruits) was not sufficient to destroy a significant correlation between abundances at those stages, but the large variability in the fraction of fish surviving from prerecruits to recruits did destroy any correlation between abundance at earlier life stages and age 1 recruitment. Therefore, recruitment strength is not determined at the early life stages.

As originally stated, Hjort's and Lasker's hypotheses about sources of interannual variation in recruitment referred

TABLE 5. Correlations, r, between reconstructed abundances at early life stages and subsequent age 1 recruitment from that same cohort for the central population of northern anchovy. In each correlation, the $\log_e$ was taken of both abundance variables. P is the probability for the null hypothesis H_0: r = 0, and sample size = 13 yr in all cases. Average age since spawning is given in parentheses.

Beginning stage	Ending stage	r	P
Eggs	Age 1	0.04	0.89
Yolk-sac larvae (4.5 d old)	Age 1	−0.09	0.76
Prerecruits (19-d-old larvae)	Age 1	0.07	0.82

TABLE 6. Correlations, r, between reconstructed abundances at life stages prior to recruitment for the central population of northern anchovy. $\log_e$ of both abundances were used in each correlation, and sample size = 13 yr in all cases.

Beginning stage	Ending stage	r	P
	Across one stage		
Eggs	Yolk-sac larvae (4.5 d old)	0.66	<0.015
Yolk-sac larvae (4.5 d old)	Prerecruits (19-d-old larvae)	0.91	<0.001
	Across more than one stage		
Eggs	Prerecruits (19-d-old larvae)	0.67	<0.014

TABLE 7. Statistics on instantaneous mortality rates (M) of northern anchovy, calculated from 13 yr of reconstructed abundances at successive life stages. SD_M is the standard deviation of M. The mean daily M was calculated as the mean total M from the beginning to the end of the period, divided by the duration of the period (in days).

Beginning of period	End of period	Mean duration of period (d)	Mean total M over period	SD_M	Mean daily M
Eggs	Yolk-sac larvae	4.4	1.584	0.544	0.360
Yolk-sac larvae	Prerecruits	14.6	2.711	0.343	0.186
Prerecruits	Age 1 recruits	346	6.378	1.170	0.018

specifically to the importance of the early larval stage, within a few days of yolk-sac absorption. However, for the anchovy population analyzed here, the early larval stage averages only 14.5 d in duration, which is only a small portion of the total duration as larvae (about 20%; Hunter and Coyne 1982) and a smaller portion of the egg-to-recruitment period. Based on the highly variable mortality after age 19 d reported here, it appears that the "critical period" (if one exists) is more likely to occur at a later stage than the early one discussed by Hjort and Lasker.

Our low correlation between 19-d-old larvae and age 1 abundance does not imply that the lack of correlation will also hold when abundance of prerecruits gets very low and outside the range of our data. Extremely low abundance of prerecruits could lead to significantly decreased recruitment, but our data do not permit a test of this hypothesis.

Regardless of the reason for lack of correlation between abundances of prerecruits and recruits, current data clearly show that it is not possible to forecast age 1 abundance from larval abundance at 19 d or before. If forecasts are needed, they will have to be made from population measures taken at an age between 19 and 365 d. Indeed, Methot (1986) showed that a reasonable index of age 1 recruits is the proportion of age 0 (not yet 1 yr old) fish caught by the Mexican commercial fishery during their 9th through 12th mo of life, just prior to becoming age 1 fish. The proportion of these age 0 fish reflects the size of the incoming year-class: the arcsine or angular transform (Sokal and Rohlf 1969, p. 386) of the proportion of these age 0 fish is significantly correlated with the $\log_e$ of age 1 fish ($r = 0.75$, $r^2 = 0.57$, $P < 0.01$). An identical analysis of the proportion of age 0 fish in their *third* quarter of life (6–9 mo old) shows much lower correlation with age 1 fish ($r = 0.33$, $r^2 = 0.11$, $P > 0.4$), as does the relation between this third quarter index and our $\log_e$(prerecruit abundance) ($r = -0.07$). The fourth quarter correlations particularly confirm that better forecasts of recruitment will arise from samples at ages closer to age 1 than the early larval stage.

Life Table

Mean instantaneous *daily* mortality rates for the three periods described here decrease with increasing age and size of northern anchovy up to age 1 (last column of Table 7), as has been found for numerous other species (e.g. Cushing 1974; Dahlberg 1979; Peterson and Wroblewski 1984). The means of the reconstructed abundances on which the M values in Table 7 are based appear reasonable because the mean instantaneous mortality rate for each period (plus symbol in Fig. 3) agrees well with theoretical expectations and with rates observed for other fishes at a similar size (Fig. 3). Note that the mean instantaneous daily mortality rate of 0.018 for the period between prerecruits and age 1 applies over a longer time (averaging 346 d) than the larger daily mortality rates at previous stages. The net result is a large mean total instantaneous mortality over the period from prerecruits to recruits (~6.4, Table 7).

The mean daily instantaneous mortality rates for the first two periods in Table 7 are slightly different from those in Smith's (1985) summary life table: 0.25 and 0.16 instead of the 0.36 and 0.186 estimated here. This is partly because Smith's estimates were from a subset of the 13 yr used here. Our value of 0.018 for the daily mortality rate from prerecruits to age 1 recruits falls within Smith's range for that period (0.01–0.05).

General Discussion

Estimation Error

Some readers may believe that the low correlation between abundances of prerecruits and recruits (Fig. 2) is due to errors in estimation of the components of the reconstruction process, rather than due to biological reality. There are a number of lines of evidence which suggest that estimation errors are *not* the explanation for this low of correlation.

First, the correlation was still low ($r = -0.21$, $P = 0.64$) when we only included data from those years in which all components were observed, rather than estimated by filling in the long-term mean. The points included (circled dots in Fig. 2) thus had less error embedded in them than the points in the original analysis. Note that these seven relatively error-free points covered the same range in abundance of prerecruits as the original 13 (Fig. 2), yet no significant correlation emerged.

Second, imprecision in the mortality estimates prior to 19 d was probably not the cause of the low correlation between our reconstructed abundance at that age and age 1 recruits. Lo's (1985) annual mortality coefficients for the periods between eggs and 19-d-old larvae were based on a large number of ichthyoplankton tows (~1000) (Lo 1986). Also, the high correlations in Table 6 among eggs, yolk-sac larvae, and prerecruits were all statistically significant and show that variation in mortality rates due to natural and sampling variability at those early stages contributes little to the low correlations between those early stages and recruitment (all of the latter r values were near zero, Table 5).

In addition, one must assume an unreasonably large amount of estimation error in the abundance of prerecruits to show that the correlation of 0.07 found here between prerecruits and recruits was derived from a stronger true underlying correlation. Under the assumption that the $\log_e$(prerecruits) are normally distributed, we used methods described by Snedecor and Cochran (1967, p. 165) to reestimate the correlation coefficient with estimation error removed from the prerecruit abundance. With parameters estimated from the correlation of $\log_e$(recruits) on $\log_e$(prerecruits), extremely large estimation errors are required to strengthen the correlation. For example, if estimation error accounts for 50% of the total variation in $\log_e$(prerecruits), the correlation coefficient only increases from 0.07 to 0.10; if it accounts for 80% of the variance in X, r is only increased to 0.16. Therefore, we conclude that estimation error alone is not sufficient to give rise to the low correlation that we observed.

Finally, the estimates of age 1 abundance appear precise. The time series of abundance of recruits which was used here was from one VPA based on one set of assumptions used by Methot (1986). However, two other time series of recruits were derived using other assumptions, and all three are significantly correlated (all $r > 0.83$, Methot 1986). In part, this consistency among the different time series of recruits arose because Methot (1986) used in his VPA four independent data sets on age compositions, which were in turn highly correlated (r values ranged from 0.61 to 0.99). Thus, it is unlikely that imprecise estimates of age 1 abundance are the cause of the lack of correlation between prerecruits and age 1 recruits.

Given these reasons, estimation error does not appear to be an important factor in our results. The lack of correlation between prerecruits and age 1 recruits in northern anchovy appears real and it is caused by the highly variable survival rate during that lengthy period.

TABLE 8. Statistics on proportion of northern anchovy cohorts surviving. Following standard formulae for lognormal distributions (Aitchison and Brown 1966), we calculated these statistics for survival rates (proportions) from the mean, μ, and variance, σ^2, of the corresponding instantaneous mortality rate (M) data shown in Table 7. Median of the 13 yr of survival rates was $e^{-\mu}$, their mean was $e^{(-\mu+(\sigma^2/2))}$, and their coefficient of variation (CV) was $(e^{\sigma^2}-1)^{0.5}$. Last column shows mean fraction of initial eggs which survived through to the end of the period indicated.

Beginning of period	End of period	Mean duration of period (d)	Median survival rate over period	Mean survival rate over period	CV	Mean daily survival rate	Mean fraction of eggs
Eggs	Yolk-sac larvae	4.4	0.205	0.238	0.587	0.722	0.238
Yolk-sac larvae	Prerecruits	14.6	0.066	0.070	0.353	0.833	1.67×10^{-2}
Prerecruits	Age 1 recruits	346	1.7×10^{-3}	3.4×10^{-3}	1.712	0.984	5.66×10^{-5}

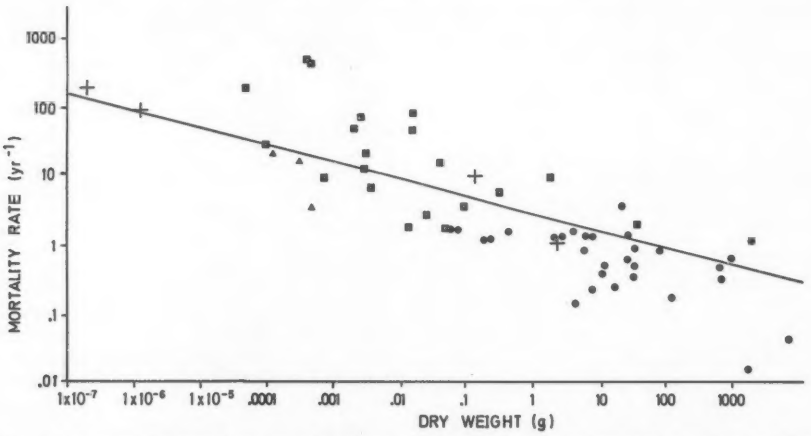

FIG. 3. Instantaneous mortality rates of different species of fish and chaetognaths in relation to body weight. Plus symbols are for the three early life stages of northern anchovy discussed here, plus anchovy adults. All data except northern anchovy are from Peterson and Wroblewski (1984). Mean body weight of northern anchovy during each of the three early life history periods in the life table was the weighted average (weighted by abundance) calculated from a survivorship curve derived from our estimated instantaneous daily mortality rate for that period (shown in Table 7) and a growth curve. We used Hunter's (1976) growth curve of dry weight vs. age for eggs to 19-d-old larvae, and from then until age 1, we used Methot's (1981) observed lengths at age converted to dry weight by his length–weight relationship.

We concur with May (1974) and W. C. Leggett (pers. comm.) that there is evidence to doubt the generality of Hjort's hypothesis that mortality of first-feeding larvae is a strong determinant of year-class strength. If we are to improve our understanding of causes of variability in recruitment of northern anchovy, research must be done on all life stages, especially those closer to age 1. Methot's (1986) correlations between recruits and proportion of age 0 fish in the Mexican catch underscore the importance of understanding the sources of variability in mortality even during the last 3 mo prior to recruitment as age 1 fish. Although the majority (57%) of interannual variability in age 1 abundance is accounted for by that fourth quarter index, there still is a large amount (43%) of unexplained variability in that period.

Other Species

Much of the research on the early life history of northern anchovy has been directed toward back-estimation of spawning biomass (Lasker 1985). But in addition, the CalCOFI program has been useful from the viewpoint of providing data to rigorously test hypotheses about sources of variability in recruitment of northern anchovy. However, it is not possible to extend our conclusions to other marine fish species with pelagic larvae because they differ from northern anchovy in the physical habitat and the species complexes of predators, competitors, and food organisms. Such differences could lead to differences in the mechanisms that cause variability in recruitment. However, to the extent that fish larvae of similar size face similar problems everywhere, our results suggest that early larval sampling programs alone will provide only a partial understanding of sources of variation in recruitment.

The question concerning the general appropriateness of data on early larval abundances alone can be answered partly by examining the literature that reports analyses of correlations between abundance of recruits and abundance at some earlier life stage. Such correlations are normally used to forecast recruitment for management purposes. Therefore, the summary

TABLE 9. Summary of reported correlations between abundances at early and later life stages. Sizes of fish, when reported, are given in parentheses below the age. References: A, Cushing (1974); B, Bannister (1978); C, Rauck and Zijlstra (1978); D, Tormosova (1980); E, Postuma and Zijlstra (1974); F, Burd (1985).

Species	Beginning stage	Ending stage	*N* (yr)	Correlation	*P*	Reference
Baltic cod	30-d-old larvae	Age 1 group	6	0.81	0.05	A
	Age 0 group	Age 1 group	6	0.90	0.015	A
North Sea plaice	Eggs	Age 2	10	0.25	0.49	B
	Age 0 group	Age 2	14	0.71	0.005	C
North Sea sole	Age 0 group	Age 2		Low		C
	Age 1 group	Age 2	12	0.68	0.13	C
North Sea haddock	First-feeding larvae (≥6 mm)	Age 2	7	0.60	0.15	D
North Sea herring						
Downs stock	30-d-old larvae	Age 3	15	0.34	0.22	E
	30-d-old larvae	Age 0	13	0.76	<0.01	F
Doggerbank stock	Small larvae (<11 mm)	Age 3	9	0.58	0.10	E
	Large larvae (≥11 mm)	Age 3	9	0.71	0.025	E
Bank stock	20-d larvae	Age 0	11	0.66	0.027	F

of published correlations in Table 9 may be biased towards significant cases because nonsignificant results would be less likely to be reported in the literature. Even so, results in Table 9 are diverse; some stocks show significant correlations and others do not. This diversity of results emphasizes the varied effect of different physical and biological processes on the relative variability in mortality at early and late life stages. Future investigations could fruitfully use a comparative approach (Bakun 1986) to determine which habitat factors are more variable for species such as northern anchovy, which show a low correlation between early larval abundance and recruitment, than for species showing a high correlation.

Table 9 also supports the intuitive reasoning that the closer that abundance samples are taken in time, the more likely a significant correlation is to exist between those abundances. This is demonstrated by Baltic cod (*Gadus morhua*), North Sea plaice (*Pleuronectes platessa*), North Sea haddock, (*Gadus aeglefinus*), North Sea sole (*Solea solea*), Downs herring (*Clupea harengus*), and Doggerbank herring (Table 9). For example, the Downs herring stock in the North Sea showed a lower correlation between abundance of 1-mo-old larvae and an index of recruitment at age 3 (Postuma and Zijlstra 1974) than was observed between those larvae and an index of age 0 fish. Such evidence supports our finding that in the northern anchovy, abundance of age 1 recruits may be better correlated with abundance at some stage closer to age 1 than the currently sampled 19-d-old larvae.

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Growth Patterns in Bluegill (*Lepomis macrochirus*) and Pumpkinseed (*L. gibbosus*) Sunfish: Environmental Variation and the Importance of Ontogenetic Niche Shifts[1]

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Osenberg, C. W., E. E. Werner, G. G. Mittelbach, and D. J. Hall. 1988. Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish: environmental variation and the importance of ontogenetic niche shifts. Can. J. Fish. Aquat. Sci. 45: 17–26.

Size-specific growth rates were determined for bluegill (*Lepomis macrochirus*) and pumpkinseed (*L. gibbosus*) sunfish collected between 1978 and 1985 in nine lakes in southwestern Michigan. Variation in growth rates was attributable to lake effects as well as an interaction between lake and year effects. Year effects explained none of the observed variation, suggesting that growth rates were influenced more by unique lake differences than by annual climatic differences. Analyses of the covariation in growth among different size-classes of bluegill and pumpkinseed revealed that small bluegill (<55 mm standard length (SL)) and small pumpkinseed (<40 mm SL) exhibited similar responses to environmental factors, while large bluegill (>55 mm SL) and large pumpkinseed (>50 mm SL) responded differently. These breaks in the growth patterns coincide with the sizes at which each species exhibits an ontogenetic shift in diet. Comparison of growth rates and resource densities suggests that the growth rates of the large fishes were food limited. Small fishes showed significant density-dependent growth. This correlative evidence for competition is in agreement with recent experimental work. We suggest that the competition between juvenile sunfishes is driven by the effects of adult resources on adult performance and the eventual recruitment of juveniles into the littoral habitat.

Les auteurs ont étudié les taux de croissance liés à la taille chez le crapet arlequin (*Lepomis macrochirus*) et le crapet-soleil (*L. gibbosus*) capturés de 1978 à 1985 dans neuf lacs du sud-ouest du Michigan. La variation des taux de croissance a été imputée à l'incidence du milieu ainsi qu'à l'interaction entre l'incidence du milieu et de l'année. L'incidence de l'année n'a pas expliqué la variation observée, ce qui porte à croire que les taux de croissance sont plus touchés par les différences du milieu que par les différences climatiques annuelles. Les analyses de la covariation de la croissance chez les différentes classes de taille du crapet arlequin et du crapet-soleil ont révélé que les petits crapets arlequin (<55 mm de longueur standard (LS)) et les petits crapets-soleil (<40 mm LS) réagissent de la même manière aux facteurs environnementaux; par contre, les gros crapets arlequin (>55 mm LS) et les gros crapets-soleil (>50 mm LS) ont réagi d'une façon différente. Ces trouées dans les régimes de croissance coïncident à la taille à laquelle chaque espèce montre un déplacement ontogénique du régime alimentaire. Une comparaison des taux de croissance et des densités des ressources porte à croire que les taux de croissance des gros poissons sont limités par la disponibilité de la nourriture. Les petits poissons montrent un taux significatif de croissance liée à la densité. Cette preuve corrélative de compétition concorde avec de récents travaux expérimentaux. Selon les auteurs, la compétition entre les crapets juvéniles est entraînée par l'incidence des ressources alimentaires des adultes sur le rendement des adultes et le recrutement éventuel de juvéniles dans l'habitat littoral.

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The study and description of growth rates in fishes have long attracted the attention of ecologists (e.g. Le Cren 1958; Gerking 1966; Brett 1979; Ricker 1979). The study of dietary patterns has attracted similar attention and has expanded from the description of general patterns of resource use among species to investigations of the shifts in diet and habitat use that arise during fishes' ontogenies (Crossman and Larkin 1959; Keast 1978; Mittelbach 1984). These ontogenetic niche shifts often occur at particular sizes in a fish's life history due to the important effect size has in determining, for example, feeding ability and vulnerability to predators (Werner 1986). The description of growth rates has typically proceeded without reference to these ontogenetic niche shifts (but see Larkin et al. (1957) and Crossman and Larkin (1959) for notable exceptions). Application of a single growth equation to the entire life history is likely to be in error if diet and habitat use, which can change abruptly during the fish's life history, play important roles in determining growth trajectories (Larkin et al. 1957). Because diet and habitat use can affect growth rates, and because growth trajectories can themselves influence the timing of the niche shifts, growth and resource utilization must be viewed from similar perspectives (Werner and Gilliam 1984). When integrated into a similar framework, studies of growth and resource utilization can lead to a more comprehensive understanding of population and community level patterns and the processes that regulate them.

In this paper, we explore variation in size-specific growth rates for two common sunfish (Centrarchidae), the bluegill (*Lepomis macrochirus*) and the pumpkinseed (*L. gibbosus*). We first examine the relative contributions of lake-to-lake and year-to-year variation in growth rates and then use this variation to examine how different size-classes respond to their environments. In our previous studies of the habitat and resource utilizations of these species in small Michigan Lakes, we found that each species exhibits a discrete shift in diet during development. Postlarval bluegill shift from feeding on littoral prey to feeding extensively on cladocerans that dwell in the open water (Mittelbach 1981, 1984; Werner and Hall 1988). Small pumpkinseed have diets similar to small bluegill, but larger pumpkinseed feed primarily on gastropods (Mittelbach 1984). These patterns of resource use suggest that the postlarval life histories of each species can be separated into two distinct stages, with small bluegill and small pumpkinseed overlapping considerably in diet, while large bluegill and pumpkinseed utilize separate resources (Mittelbach 1984). In this paper, we show that these patterns in resource utilizations have direct consequences to the size-specific growth rates of fish of each species. Additionally, we explore the patterns of density dependence in growth rates and provide evidence suggesting that the growth rates of small and large fish of both species are food limited. Finally, we discuss the implications of these findings to species interactions among size-structured populations.

Methods and Results

The Lakes

The nine lakes used in this study are located within 40 km of the Kellogg Biological Station in southwestern Michigan, USA. Many of the data reported in this paper were collected as part of previous or ongoing studies of the fish populations inhabiting these lakes (e.g. Hall and Werner 1977; Werner et al. 1977; Laughlin and Werner 1980; Mittelbach 1981, 1983, 1984; Werner and Hall 1988; C. W. Osenberg, unpubl. data). The selection of lakes was based on different criteria in each of the previous studies, but in no case was a lake selected on the basis of fishes' growth rates. The lakes are similar in many of their general physical and biological characteristics (Table 1) and represent a relatively homogeneous set of waterbodies compared with the large diversity available within the vicinity. The lakes are all small (5–26 ha), hardwater lakes with maximum depths between 10 and 16 m, with the exception of Three Lakes III (maximum depth = 4 m). Except for Three Lakes III, the lakes thermally stratify during the summer, and the depth of the epilimnion (3.5–5 m) generally coincides with the lower distribution of macrophytes.

The fish community that inhabits these lakes is typical of small glacial lakes in the northcentral United States. Centrarchids (notably bluegill and largemouth bass (*Micropterus salmoides*)) comprise well over half of the total fish biomass (Brown and Ball 1942; Hall and Werner 1977; Werner et al. 1977), although they may sometimes be outnumbered by cyprinids, who due to their small size comprise only a small fraction of the total fish biomass. Members of other families (e.g. Amiidae, Cyprinodontidae, Ictaluridae, Percidae) are typically present but in low abundance, although the yellow perch (*Perca flavescens*) is sometimes common.

Calculation of Growth Rates

Between 1978 and 1985, scale samples were collected from 3370 bluegill and 742 pumpkinseed inhabiting the study lakes (see Table 1). Fish were seined and/or angled between April and September, and standard lengths (SL) were recorded to the nearest millimetre. Scales were collected from the left side of each fish just posterior of the tip of the depressed pectoral fin. Dried scales were pressed onto acetate sheets, and the images were projected with a microfiche viewer. The distances from the focus to each annulus and the scale margin were measured and converted to the nearest 0.01 mm. Only nonregenerated scales were used for analysis, and only one scale was read per fish.

Back-calculations of fish size were determined using the Fraser–Lee method (Tesch 1968):

$$(1)\quad SL_x = (SL_c - SL_0)R_x/R_c + SL_0$$

where SL_x is the estimate of the standard length at age x, SL_c is the standard length at capture, and R_c and R_x are the scale radii at capture and at age x. To estimate SL_0, we examined the relationship between fish length (SL_c) and scale radius (R_c) for data pooled across all lakes and collection dates for each species. The relationship for each species was well-described by a linear function:

$$(2a)\quad \text{Bluegill: } SL_c = 11.9 + (38.3)R_c,\ n = 3334,\ r^2 = 0.97$$
$$(2b)\quad \text{Pumpkinseed: } SL_c = 11.5 + (34.1)R_c,\ n = 742,\ r^2 = 0.93$$

where the intercepts (11.9, 11.5) are the best estimates of SL_0.

Standard lengths were converted to wet masses (M in grams) based on regressions of mass on length:

$$(3a)\quad \text{Bluegill: } M = 0.00001433(SL^{3.202}),\ n = 161,\ r^2 = 0.996$$
$$(3b)\quad \text{Pumpkinseed: } M = 0.00001529(SL^{3.224}),\ n = 116,\ r^2 = 0.996.$$

TABLE 1. Description of the nine study lakes.

Lake	Location	Area (ha)	Maximum depth (m)	Midsummer thermal stratification?	pH[a]	Alkalinity[a,b]	Dominant littoral substrata[c]	D
Bassett	42°39′50″N 85°29′03″W	18	12	Yes	8.3	138	Bare sediment and debris	Lm
Culver	42°27′30″N 85°14′08″W	13	12	Yes	—	—	*Chara*	Lm
Deep	42°37′10″N 85°27′34″W	13	11	Yes	8.3	135	*Chara*	Lm
Hamilton	42°24′22″N 85°18′48″W	16	13	Yes	—	—	*Chara*	Lm
Lawrence	42°26′27″N 85°21′00″W	5	13	Yes	8.3	196	*Scirpus subterminalis*	Lm
Palmatier	42°34′19″N 85°26′16″W	6	12	Yes	8.4	136	*Chara*	Lm
Three Lakes II	42°21′07″N 85°25′55″W	22	10	Yes	8.2	160	*Chara*	Lm
Three Lakes III	42°21′17″N 85°25′28″W	15	4	No	—	—	*Chara*, *Myriophyllum*, *Potamogeton*	Lp
Warner	42°28′17″N 85°31′26″W	26	16	Yes	8.5	100	Bare sediment and debris, *Chara*, *Potamogeton*	Lm

[a]Unpublished data provided by Roger Bachmann, Iowa State University. — = no data.
[b]$CaCO_3$ equivalent (mg/L).
[c]Dominant littoral substrata ranked by relative abundance.
[d]Dominant fish species ranked by relative abundance. Lm = *Lepomis macrochirus*; Ms = *Micropterus sal*

Dominant fish species[a]	Years of collection (number of fish)	
	Bluegill	Pumpkinseed
Lm, Pf, Ms	1978	—
	(25)	(0)
Lm, Ms	1985	1984–85
	(173)	(31)
Lm, Ms	1982, 1984	1982, 1984
	(598)	(76)
Lm, Ms	1982	—
	(197)	(0)
Lm, Ms	1978–79, 1981–82, 1984–85	1981, 1984–85
	(721)	(100)
Lm, Ms	1985	1984–85
	(183)	(98)
Lm, Ms, Lg	1978, 1980–82, 1984–85	1980–81, 1984–85
	(815)	(288)
Lg, Lm, Ms	1981	1981, 1984–85
	(82)	(72)
Lm, Ms	1982, 1984	1982, 1984
	(576)	(77)

erus salmoides; Lg = *Lepomis gibbosus*; Pf = *Perca flavescens*.

Annual growth rates (i.e. the change in size from one annulus to the next) were expressed in one of two ways using equations 1–3:

(4a) Change in mass: $\Delta M = M_{x+1} - M_x$
(4b) Change in length: $\Delta SL = SL_{x+1} - SL_x$.

Growth rates were expressed as functions of the fish size at the start of the year's growth stanza (i.e. M_x or SL_x). For most of the data presentations and analyses, fish were divided into 5-mm size-classes. For each species, we restricted our analyses to the 15 size-classes between 20 and 95 mm SL due to low sample sizes for other size-classes.

Validation of Scale Technique

We tested our ability to distinguish annuli accurately by comparing our estimates of age based on the number of annuli with independent estimates of age based on size–frequency distributions of collected fish. Three collections of bluegill provided sufficient resolution to distinguish at least three (at most five) age-classes from the size–frequency distributions. Of the 328 bluegill falling clearly into one of the size–frequency age-classes, only 8 were "misclassified" based on their number of annuli. Misclassifications were more common in older fish, and all "errors" could be attributed to overlapping size distributions of the age-classes. Additionally, if we compare the mean size of fish in each of the age-classes using the two techniques (which tends to smooth out the problems with the overlapping distribution), the two techniques yield almost identical results ($r = 1.00$, $n = 11$; slope not different from 1.00 at $p > 0.20$). This analysis showed that we could successfully identify the first through fifth annuli for fish up to 125 mm SL, which is beyond the size limit for the fish used in this paper.

IN doing our analyses, we discovered that the readings of some of the bluegill and pumpkinseed scales used in two previous studies (Mittelbach 1983, 1984) included extra marks, perhaps spawning checks. Before being used in the present study, the scales were reread by the same person that read most of the other scales. We mention this problem primarily to clarify differences in growth rates reported here with those in Mittelbach (1983, 1984). Reanalysis based on the new readings show that conclusions in Mittelbach (1983, 1984) were unaffected by the rereadings.

Lake and Year Variation in Growth

Variation in growth can be caused by a number of factors, and these factors might operate in different ways depending on their spatial and temporal scales. We estimated the relative importance of lake and year variation in growth rates by analyzing the growth ($\log_{10}(\Delta SL)$) for each of the 30 size-classes with a random effects model of analysis of variance (SAS Institute Inc. 1985: Proc GLM, type III sums of squares). For each of the 30 analyses, we first tested whether the model accounted for a significant portion of the variation; then we estimated the relative importance of each source of variation by partitioning the total variance into its four component variances (error, lake, year, and lake × year). The data were unbalanced and all designs contained missing cells, which prevented the total variance from equaling the sum of the components. We observed no clear pattern in the variance partitioning with fish size or species, so we present the results as means and standard errors taken over all of the separate analyses.

Of the 30 analyses, 28 allowed estimation of all four variance components. Among these 28 analyses, 19 of the models explained a significant fraction of the observed variation (each analysis evaluated at $p < 0.05$), although the error variance averaged 69% of the total variance (SE = 3.7), $n = 28$). The large error component probably reflects variation in our sampling technique (e.g. nonrandom fish collections and variation in our scale techniques) as well as individual differences among fish within a size-class (e.g. due to genetics, sex, age, and parasite loads). Variation among lakes accounted for an average of 20% (SE = 7.2) of the total variance. Year effects, however, did not explain any of the variation ($s^2_Y/s^2_T = -1\%$, SE = 2.9: the negative value arising due to the unbalanced data). Instead, the remaining variation in growth rates was in the form of unique yearly fluctuations within lakes ($s^2_{L\cdot Y}/s^2_T = 20\%$, SE = 5.9). Very similar results were obtained using ΔSL, $\log_{10}(\Delta M)$, or ΔM as the response variable, suggesting that the relative strengths of the variance components were not simply a scaling problem.

Correlated Responses of Different Size-Classes

Variation in the environment can produce a variety of effects on the growth of fishes of different species or size-classes. For example, variation in temperature would probably have the same qualitative effect on fishes of all sizes (and probably of different species) because of the similar effect of temperature on the fishes' metabolic rates and activity levels. Similarly, if food abundance varied (and was limiting), and fishes of different species or size-classes fed on the same food, then the growth rates of the fishes would be correlated across these different food environments. However, if the fishes fed on different types of food, then their growth rates could covary negatively, or positively, or be uncorrelated, depending on the pattern of covariation among the different prey types. Thus the patterns of covariation in growth rates across different environments can potentially be used to classify fishes into categories that are ecological similar in their response to variation in important environmental parameters.

To examine the similarities in growth between different size-classes of bluegill and pumpkinseed, we determined the mean growth rate (ΔSL) for each size-class in each lake and year of growth (i.e. "environment"). The results from the analyses of variance revealed that as much variation was observed within a lake across years as was observed among lakes. Therefore, we analyzed the widest range of environments by examining growth in each combination of lakes and years. We discarded growth rate estimates that were based on only one observation. From these means (average sample size = 15), we calculated the correlations among the growth rates for each size-class with the growth rates for each of the other size-classes. This yielded 435 (=[30·30 − 30]/2) correlation coefficients among the 30 size-classes (see Fig. 1 for two examples). Sample sizes for the correlations were ≤42 (mean = 11), with the smaller sample sizes associated with correlations among large size-classes.

In order to summarize and interpret this large correlation matrix, we performed a principal factor analysis (SAS Institute Inc. 1985: Proc factor). Each factor can be conceptualized as representing a particular, but unspecified, environmental factor that caused variation in the growth rates. The association between the factor and the growth rate of a size-class is represented by the correlation (i.e. loading) of the variable with the factor. Thus, the analysis can be used to reveal which size-classes respond similarly to environmental variation by comparing the similarility in loading patterns among the size-classes. We extracted two factors and rotated them using the

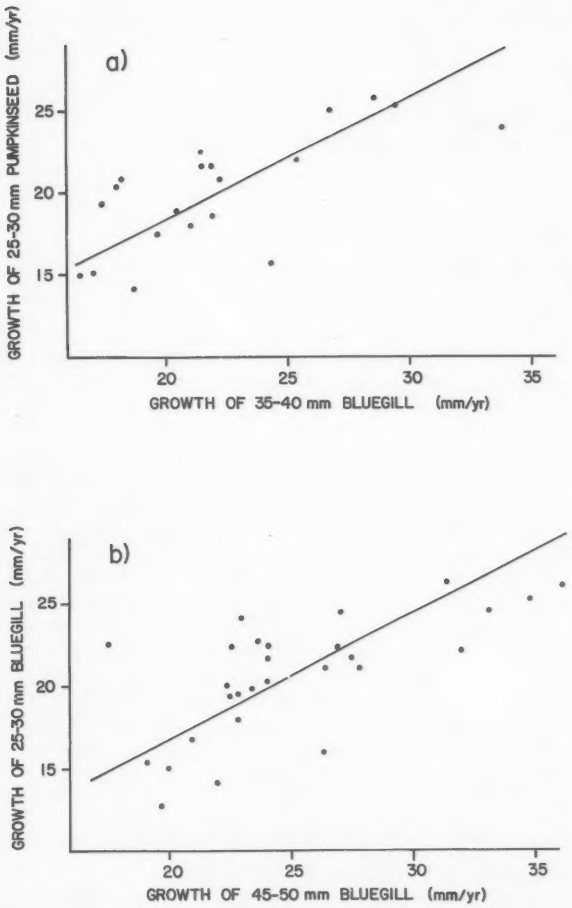

FIG. 1. Growth rate covariation among size-classes of bluegill and pumpkinseed sunfish. (a) $r = 0.72$, $n = 20$, $p < 0.01$; (b) $r = 0.68$, $n = 28$, $p < 0.01$.

quartimax technique. The rotation affects only the quantitative values of the loading patterns; the relative patterns of the size-classes are unaffected. The first two factors explained over 60% of the total variance, and subsequent factors contributed relatively little to the explained variance because each tended to be uniquely associated with only one or a few of the size-classes. Because the analysis was derived from the correlation matrix, whose elements were based on different sample sizes, the two factors could theoretically account for greater than 100% of the observed variation in some of the variables.

The factor analysis explained a substantial portion of the variation in growth for many of the size-classes and provided clear demarcation between small and large size-classes of each species (Table 2). This demarcation is seen most clearly in Fig. 2a and 2b in which the factor loadings are plotted for each of the size-classes. Notice also how the small size-classes of bluegill loaded similarly as the small size-classes of pumpkinseed. In particular, the results show that the responses of similar-sized fishes of *different* species were more similar than the responses of small and large fish of the *same* species. Based on the loading patterns, we defined two life history categories for each species containing the consecutive size-classes that exhibited similar

TABLE 2. Rotated factor loading patterns for the growth rates of the 30 size-classes. R^2 is the variance explained by the two factors for each variable and is equal to the sum of the squared loadings on each factor. Variance explained by factor 1 = 43%; variance explained by factor 2 = 22%.

Size-class (mm)	Bluegill			Pumpkinseed		
	Factor 1	Factor 2	R^2	Factor 1	Factor 2	R^2
20–25	+0.72	−0.17	0.55	+0.75	+0.23	0.62
25–30	+0.85	−0.04	0.72	+0.76	−0.27	0.65
30–35	+0.81	+0.09	0.66	+0.51	−0.42	0.44
35–40	+0.91	+0.20	0.87	+0.70	−0.13	0.51
40–45	+0.85	+0.31	0.82	−0.09	+0.44	0.20
45–50	+0.93	+0.56	1.18	+0.50	−0.12	0.26
50–55	+0.74	+0.49	0.79	−0.35	+0.33	0.23
55–60	+0.27	+0.84	0.78	−0.22	+0.44	0.24
60–65	+0.27	+0.73	0.61	−0.58	+0.61	0.71
65–70	−0.14	+1.00	1.02	−0.95	+0.19	0.94
70–75	−0.43	+0.13	0.20	−0.80	+0.25	0.70
75–80	−0.21	+0.44	0.24	−0.89	+0.66	1.23
80–85	−0.04	+0.35	0.12	−0.94	+0.73	1.42
85–90	0.00	+0.82	0.67	−0.95	+0.20	0.94
90–95	−0.34	+0.81	0.77	−0.14	+0.53	0.30

growth rate responses to the environmental variation: 20–55 mm bluegill, 55– 95 mm bluegill, 20–40 mm pumpkinseed, and 50–95 mm pumpkinseed (Fig. 2). The 40–50 mm pumpkinseed provided ambiguous results.

Growth Rates and Fish Densities

The factor analysis, as well as our previous studies of diet and habitat use, suggests that three functional categories of fishes can be defined: small fishes (bluegill and pumpkinseed combined) which grow similarly and feed on soft-bodied littoral prey; large bluegill which feed extensively on zooplankton; and large pumpkinseed which feed primarily on snails. In this section, we compare the densities of these three categories with the growth rates of small and large bluegill and small and large pumpkinseed.

Fish densities were estimated from underwater transect counts conducted in seven of the nine lakes between 1983 and 1985. Fish were counted by one to three divers swimming parallel to shore. The divers swam new positions along the transect until all parts of the littoral zone and nearby limnetic areas had been censused (see Werner et al. (1977) and Hall and Werner (1977) for a description of the general technique). Transect counts were done during midday when large bluegill are absent from offshore limnetic areas (Werner et al. 1977). Minimum visibility was >2.0 m on all dates. All observed bluegill and pumpkinseed, except for young-of-the-year, were counted and placed into two size-classes (<75 and >75 mm SL), which roughly correspond to the two life history categories defined by resource use and growth patterns. Densities of small fishes and large pumpkinseed were expressed as the number of fish per 100 m² of littoral zone. Densities of large bluegill were expressed as the number of fish per 100 m² of total lake area because, in these lakes, large bluegill feed predominately on limnetic zooplankton and, to a lesser extent, littoral prey. However, since littoral area and total lake area are significantly correlated among these lakes ($r = 0.83$, $n = 7$, $p < 0.05$), the rankings of lakes by density for any of the fish categories are robust to the exact method used in calculating density. The estimates, especially for the small fish, may underestimate the

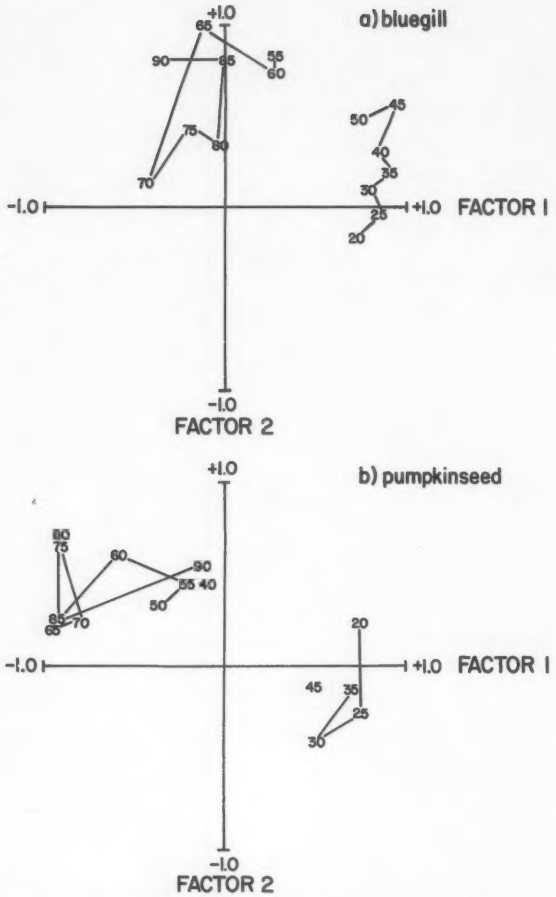

FIG. 2. Factor loadings for size-classes of bluegill and pumpkinseed sunfish. The loadings (correlations) of each size-class with the two factors are shown. Solid lines connect adjacent size-classes that are defined a posteriori to comprise a life history category. The loadings for the two species were plotted in separate figures for clarity.

absolute densities (cf. Mittelbach 1986, 1988); however, the counts are quite repeatable within a season (Hall and Werner 1977; Werner et al. 1977) and probably provide accurate relative abundance estimates across the lakes.

For each lake, the growth data from the year(s) during which the transect counts were made were pooled with the growth data from the immediately preceding and following years, although data were not always available from all of these years. We pooled growth data from adjacent years in order to increase the sample sizes. Our observations suggest that although densities have changed over a 10-yr period in several of these lakes, year-to-year changes are relatively small, due perhaps to the generation times of these fish ($\geq$3 yr). Variation in densities (or other factors that affect growth) during these 3-yr periods would increase the error variance in the analysis of the relationship between growth and density; any patterns that emerge do so in spite of the added noise. In Three Lakes III, we lacked growth data for bluegill for the entire 3-yr period. In this case, we used bluegill growth rates for all available years and associated them with the densities estimated in 1983. Qualitative transect counts from 1981 show bluegill densities in Three Lakes III to be very low (Mittelbach 1984), which is consistent with the pattern observed in 1983.

We derived estimates of fish growth for small and large bluegill and small and large pumpkinseed by performing four separate analyses of covariance using lakes as the grouping variable, $\log_{10}(\Delta M)$ as the dependent variable, and $\log_{10}(M_x)$ as the covariate. The transformations successfully linearized the relationships, and the slopes were homogeneous among lakes in all analyses except the one for small bluegill. Inspection of the relationships showed that the heterogeneity within the small bluegill category was relatively minor and did not affect our conclusions about the general effects of lakes on small bluegill growth rates. Adjusted means from each of the four analyses were back-transformed to provide quantitative estimates of growth rates for the four fish categories in each of the seven lakes from which we also had estimates of fish densities (Table 3).

Correlations were obtained for each combination of the growth and density variables (Table 4). As suggested by the factor analysis, the growth rates of the small bluegill and pumpkinseed were strongly positively correlated, and the growth responses between the same sized fishes of different species were more similar than the growth responses of different size categories of the same species. Furthermore, the growth rates of small fishes of both species were negatively correlated with the combined density of small fishes (Fig. 3). Interestingly, large pumpkinseed and large bluegill did not exhibit negative density dependence as seen for the small fishes; their respective growth rates and densities were positively correlated. The density of large bluegill and the density of small fishes were significantly positively correlated, due to the high fraction of the small fish category comprised by small bluegill.

Bluegill Growth Rates and Habitat Profitability

In small Michigan lakes, large bluegill feed extensively on zooplankton, primarily *Daphnia* (Mittelbach 1981, 1984; Werner and Hall 1988). Therefore, we used estimates of the zooplankton abundances in the study lakes to examine whether the growth rates of large bluegill were positively related to resource levels. Zooplankton were sampled on five dates between May and August 1983 in four of the lakes (Deep and Lawrence lakes and Three Lakes II and III). The *Daphnia* in these lakes exhibit diel vertical migration (Haney and Hall 1975), and the bluegill feed on these prey primarily during the brief periods at dawn and dusk during which the zooplankton are in the epilimnion and there is sufficient light to permit feeding by the bluegill. Therefore, sampling was always done within 15 min of sunrise. Three vertical tows were taken from a depth of 4 m (3 m in Three Lakes III) in the limnetic zones of each lake. The three samples were pooled and the *Daphnia* were counted and measured. From the zooplankton densities and size–frequency distributions, net foraging rates were estimated using the model developed by Mittelbach (1981). The estimated foraging rates, or habitat profitabilities, were calculated for a 100-mm SL bluegill. The absolute magnitude of these estimates vary depending on the fish size (see Mittelbach 1981), but the ranking of lakes is extremely robust. The rankings of average profitabilities and large bluegill growth rates were perfectly correlated (Table 5, Spearman rank correlation: $r_s = 1.00$, $n = 4$, $p < 0.05$), suggesting that the growth rates of large bluegill were food limited. Interestingly, there was also a perfect rank correlation between bluegill densities and zooplankton profitabilities (Table 5). In

TABLE 3. Growth rates and fish densities in seven lakes. Growth rates are the adjusted means from analyses of covariance (see text). Sample sizes are given parenthetically. Fish densities are expressed as per area of littoral zone for small fish and large pumpkinseed and as per total lake area for large bluegill. Small fish densities are the combined densities of small bluegill and small pumpkinseed. See text for sizes of small and large fish.

Lake	Years used in estimation of:		Adjusted growth rates (g/y)				Densities (no./100 m²)		
			Bluegill		Pumpkinseed				
	densities	growth rates	Small	Large	Small	Large	Small fish	Large bluegill	Large pumpkinseed
Culver	1985	1984	3.75 (150)	18.8 (4)	3.03 (2)	15.7 (6)	16.3	2.72	1.24
Deep	1983–84	1982–83	4.71 (383)	25.1 (44)	3.26 (10)	20.4 (9)	12.0	3.96	0.93
Lawrence	1983–84	1982–84	4.43 (276)	22.3 (32)	3.12 (28)	18.5 (15)	9.0	1.79	1.01
Palmatier	1984	1983–84	4.79 (160)	18.2 (27)	4.36 (14)	18.0 (86)	2.5	0.37	1.04
Three Lakes II	1983	1982–84	3.41 (411)	22.9 (60)	3.30 (193)	27.8 (105)	14.6	2.42	1.46
Three Lakes III	1983	1976–80[a] 1982–83[b]	5.53 (81)	16.1 (51)	4.80 (20)	21.4 (6)	1.1	0.70	2.69
Warner	1983	1982–83	2.88 (320)	20.2 (145)	2.26 (15)	30.2 (14)	16.8	2.65	1.84

[a]For small and large bluegill.
[b]For small and large pumpkinseed.

TABLE 4. Correlations among growth rates and fish densities given in Table 3. Correlations $> +0.75$ or < -0.75 are significant at $p < 0.05$. The sample size for each correlation is 7.

	Small pumpkinseed growth	Large bluegill growth	Large pumpkinseed growth	Small fish density	Large pumpkinseed density	Large bluegill density
Small bluegill growth	+0.86	−0.30	−0.59	−0.88	+0.18	−0.48
Small pumpkinseed growth	—	−0.55	−0.40	−0.92	+0.36	−0.73
Large bluegill growth		—	+0.20	+0.52	−0.64	+0.76
Large pumpkinseed growth			—	+0.38	+0.39	+0.21
Small fish density				—	−0.30	+0.81
Large pumpkinseed density					—	−0.38

other words, a lake with profitable zooplankton had abundant bluegill and those bluegill grew well after reaching sizes where they could feed in the open water.

Discussion

Patterns of growth rate covariation (Table 2; Fig. 2) demonstrated almost identical demarcation among the sunfishes' life history stages as have our previous studies of diet and habitat use. Mittelbach (1984) and Werner and Hall (1988) have shown that bluegill shift their diets from littoral prey to open-water prey between 55 and 85 mm SL; the growth analysis showed a similar distinction at 55 mm SL. Pumpkinseed shift diets at slightly smaller sizes than bluegill, between 45 and 70 mm SL (Mittelbach 1984), and their growth trajectories appeared to shift between 40 and 50 mm. The diet and habitat utilizations are based on instantaneous measurements whereas the growth patterns are based on the average growth accumulated over an entire year. The lower shift points defined by the growth patterns are due to this longer reference frame; some fish that started the season feeding on soft-bodied littoral prey accumulated enough growth during the summer to conclude the season feeding on zooplankton or snails. Given this averaging effect, it is surprising that the shifts in the growth trajectories

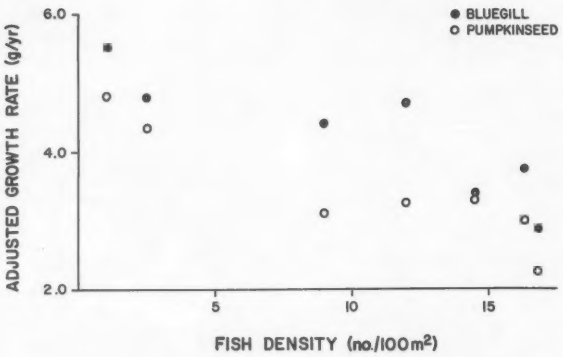

FIG. 3 Density-dependent growth of small bluegill and pumpkinseed sunfish. Growth rates and densities are from Table 3. Density is the combined density of bluegill and pumpkinseed smaller than 75 mm SL.

TABLE 5. Profitabilities (J/s) of the open water of four lakes during the summer of 1983. Estimates were derived from the model of Mittelbach (1981) for a 100-mm SL bluegill. The growth rates and densities of large bluegill were taken from Table 3.

Date	Deep	Three Lakes II	Lawrence	Three Lakes III
23–26 May	0.218	0.241	0.206	0.063
10–14 June	0.311	0.217	0.184	0.128
27 June – 1 July	0.165	0.128	0.155	0.112
16–19 July	0.209	0.136	0.160	0.092
30 July - 3 August	0.135	0.131	0.100	0.089
Seasonal mean	0.208	0.171	0.161	0.097
Large bluegill growth rate (g/yr)	25.1	22.9	22.3	16.1
Large bluegill density (no./100 m²)	3.96	2.42	1.79	0.70

were as clean as they were. The similarity among the shift points in resource utilizations and in growth patterns within a species demonstrates that resource use has important effects on the growth patterns of a species. The results also show that similar resource use by individuals of different species (e.g. small bluegill and small pumpkinseed) causes a convergence in growth.

Our characterizations of the life histories of bluegill and pumpkinseed were facilitated by the expression of both growth and resource use as functions of a common variable, body size. A large amount of data demonstrate that many aspects of an animal's ecology vary allometrically with its body size (see Peters 1983). In many cases, there are sound causative explanations of why ecological processes scale with body size. For example, body size affects prey detection (Hairston et al. 1982), handling times (Werner 1974; Mittelbach 1981, 1984), and metabolic rates (Winberg 1960) of fishes. Despite the major role of size in determining important aspects of a species' ecology, growth rates (or other variables) are often expressed as functions of age (or time) (e.g. Ricker 1979). Very different conclusions can be drawn by comparing growth curves on an age-specific rather than size-specific basis (see Larkin and Smith 1954; Larkin et al. 1957). Interestingly, the original derivations of growth equations were based on allometric scaling of growth, or physiological processes, to body size (von Bertalanffy 1957; Ricker 1979). The integration of these equations produces equations that relate size to age (i.e. time), and it is this representation of growth that is popular among many ecologists. In Fig. 4, we present growth data for bluegill from Warner Lake, Three Lakes II, and Three Lakes III to illustrate how the form of data presentation can greatly affect the interpretation of growth patterns.

In Fig. 4a, we follow the traditional approach by presenting size versus age. The classical interpretation of this figure is that bluegill grow best in Three Lakes III and worst in Warner Lake. Clearly, the fish in Three Lakes III reach all sizes at earlier times than do the fish in Warner Lake. However, these data cannot be used to infer that the environment offered by Three Lakes III is the best for all ages (or sizes) of fish, or that growth rates are higher throughout the life history in Three Lakes III. Growth rates must be inferred from the slopes of the curves in Fig. 4a. Analysis of age-specific growth rates (Fig. 4b) also leads to the conclusion that the lakes differ consistently in their ability to support fish growth; fish in Three Lakes III grow more during a year than do fish of the same age in Warner Lake. However, size and not age probably contributes more to the feeding rates and metabolic rates of fishes (Larkin et al. 1957; Brett 1979; Gerking and Raush 1979). Comparison of size-specific growth rates (Fig. 4c) indicates that a lake's "quality" depends on the size of the fish; small fish grow best in Three Lakes III but large fish grow best in Warner Lake. Figure 4b obscured this important distinction because old fish in Three Lakes III were larger than the same-aged fish in Warner Lake, and larger fish (at least in this range of fish sizes) grow faster than smaller fish. Therefore, previous growth history provided the older fish in Three Lakes III with a growth advantage despite the poorer environmental conditions under which they were living. Size-specific growth was the only presentation that distinguished the differential responses of the two life history stages of bluegill. In comparing the response of a species to a variety of environments, the most rigorous conclusions can be made by controlling for traits that alter the effects of the environment; size is probably the most important trait in fish and other organisms that vary dramatically in size, but other traits, such as age, sex, and genotype, are probably also influential. Once the primary inherent differences among the fish are controlled for, the environmental factors that were responsible for the shifts in growth trajectories and growth rates can be investigated.

One major environmental factor that can influence fish growth is water temperature (Elliot 1975a, 1975b; Craig 1982). The climatic conditions that affect water temperature in a single lake probably have similar effects on temperature in nearby lakes. Other climatic factors, such as rainfall, that occur on large scales should also produce similar effects among lakes. However, our finding that there was no appreciable yearly variation in growth rates among lakes suggests that variation in these broad climatic factors was not important in explaining the observed variation in growth rates. Instead, our results suggest that the explainable variation in growth rates was entirely due to the combined effects of lakes and unique yearly fluctuations within each lake. This suggests that exploration of the factor(s) immediately responsible for the growth rate variation can only be found by detailed examination of particular lakes.

Two important factors that vary from lake to lake that can affect growth rates are the densities of the fishes and the densities of their prey. Our survey suggests that small bluegill and pumpkinseed might compete for a limited resource (Fig. 3). Taken alone, the negative correlations between growth and density in this "natural experiment" provide only weak evidence

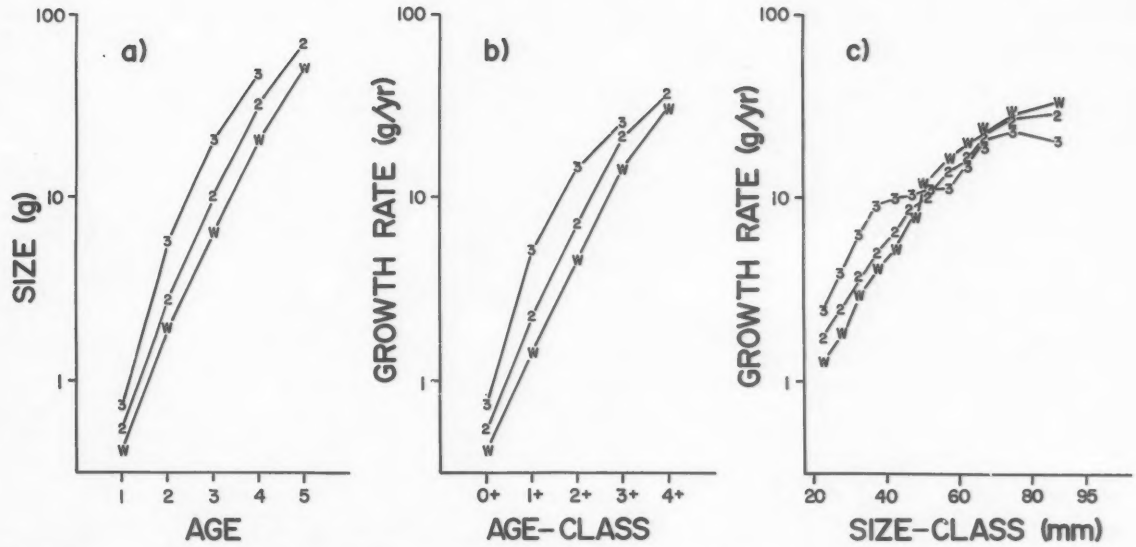

FIG. 4. Three presentations of growth curves for bluegill in three lakes: (a) size at each age; (b) age-specific growth rates; (c) size-specific growth rates. W = Warner Lake; 2 = Three Lakes II; 3 = Three Lakes III.

for competition. For example, it could be argued that a factor other than competition caused slow growth and that slow growth restricted a greater number of age-classes to the <75 mm SL size category, thus increasing the density of small fishes and producing the negative correlations. However, Mittelbach (1986, 1988) has experimentally shown that small bluegill and pumpkinseed compete exploitatively within the littoral zone of Lawrence Lake. Taken together, these data suggest that the small fishes compete for littoral prey in the study lakes and that the intensity of competition is driven by the effect of fish density on prey density and subsequent fish growth.

Adult growth rates did not show the same negative density dependence as did the growth rates of small fishes. Instead, density and growth of large fish were positively correlated (Table 5). However, our results do provide evidence that the growth rates of large fish were affected by food availability. The growth rates of large bluegill were greatest in lakes that had the highest zooplankton profitabilities (Table 5). The ranking of three of these lakes by profitabilities in a previous year (1981) was identical to the ranking in 1983: Three Lakes II (0.194 J/s) > Lawrence Lake (0.122 J/s) > Three Lakes III (0.079 J/s). (These profitabilities were derived from the data reported in Mittelbach (1984).) Mittelbach (1984) also provided seasonal estimates of snail biomasses during 1981 in these lakes. The ranking of lakes based on snail biomasses (Three Lakes II > Three Lakes III > Lawrence Lake) is in perfect agreement with the ranking of the growth rates of large pumpkinseed (Table 4). These data provide good evidence that the growth rates of large bluegill and large pumpkinseed are limited by the availability of their primary foods (zooplankton or snails). Whether the large fishes also reduce the abundances of these prey and thereby compete intraspecifically for the limited resources remains an open question.

Bluegill are commonly more abundant than pumpkinseed and other littoral feeding fishes in many lakes of North America (Brown and Ball 1942; Hall and Werner 1977; Werner et al. 1977). Svardson (1976) has also shown that planktivores dominate littoral fishes in Scandinavian lakes. The dominance of bluegill, or other planktivores, may be a result of the relatively high lakewide production of zooplankton (Svardson 1976; Werner and Hall 1979; Mittelbach 1984), due either to the greater availability of open water relative to the littoral habitat and/or possibly to a greater productivity of the zooplankton on a unit habitat basis. Relatively high zooplankton production could support planktivore densities greater than the densities of benthic feeding fishes supported by the littoral production. Only in lakes where zooplankton production is low (e.g. Three Lakes III due to the small pelagic zone and absence of a hypolimnion) can the pumpkinseed, or other littoral species, achieve greater dominance status in the fish community.

This simple scenario is complicated by two important aspects of the life histories of many fishes. The first is that small fish very often use resources and/or habitats that are discrete from those used by the adult fish of the same species. For example, in our studies, large bluegill obtain a large fraction of their diet from zooplankton, while small bluegill feed mostly on littoral prey. Although the juveniles and adults use separate habitats and/or resources, each class can indirectly influence the other by affecting the other's density, i.e. by growth of juveniles into the adult class and by production of young by adults. If the performances (measured as growth, survival, and/or fecundity) of fish are affected by intraclass competition, then increasing the productivity of adult resources, without similarly increasing the production of juvenile resources, can produce several interesting results (see Mittelbach and Chesson 1987, especially their fig. 21.4): increased density of the adult resource, increased performance of adult fish, increased densities of both juveniles and adults, and decreased performance of juvenile fish due to increased competition. The presence of the juvenile stage buffers the adult resource from a complete response by the adult consumer, leading to the positive correlations among adult density, adult performance, and adult resource density, all of which are negatively correlated with juvenile performance. The positive correlations between adult performance and den-

sity (and adult density and resource density) arise despite intraspecific competition among the adults. These results are quite different from those predicted from standard intraspecific competition models and are consistent with the patterns that we see in the bluegill and pumpkinseed systems.

However, in the bluegill–pumpkinseed system, and probably in many other systems where, for example, predators confine small fish to safe habitats, juveniles of the two species share a similar resource. If the juveniles compete for the shared resource (as is the case for small bluegill and small pumpkinseed), then the dynamics of the two species become coupled via the interaction among the juveniles (Mittelbach and Chesson 1987). The resulting dynamics are potentially complex, but variation in any of the resource productivities leads to positive correlation between the performance of adult bluegill and adult pumpkinseed and to positive correlation between the performance of juvenile bluegill and juvenile pumpkinseed. Indeed, our data show that adults (or juveniles) of the different species are more similar in their growth responses than are the adults and juveniles of the same species. Therefore, the presence of ontogenetic niche shifts (which produce the ecological distinctions among size-classes) and interspecific competition (which couples the dynamics of the interacting species) can lead to ecological patterns that are fundamentally different from those predicted by classical theory. Advancement in our understanding of aquatic systems, which are dominated by size-structured populations, will necessarily involve new approaches that focus on the interplay of body size, niche shifts, and growth rates in determining population dynamics and community level phenomena.

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Impacted Stress: A Causal Agent of Reduced Whitefish (*Coregonus lavaretus*) Egg Incubation Time

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Næsje, T. F. and B. Jonsson. 1988. Impacted stress: a causal agent of reduced whitefish (*Coregonus lavaretus*) egg incubation time. Can. J. Fish Aquat. Sci. 45: 27–31.

The purpose of these experiments was to investigate whether whitefish (*Coregonus lavaretus*) eggs subjected to induced agitation had a shorter incubation period to hatching than undisturbed incubating eggs. Eyed eggs were divided into four main groups: two incubated at river temperatures and two incubated in heated water. River temperature was 2°C at the beginning of the experiment and increased to more than 10°C at hatching. Heated water temperatures varied between 6.5 and 8.5°C. One sample with four replicates at each incubating water temperature was continuously agitated with flowing water, while two samples with four replicates each were incubated undisturbed. At both temperatures, eggs kept in motion by flowing water hatched at fewer degree-days (heated water: 380 ± 6.4, natural water: 417 ± 6.6) from fertilization to 50% hatching than those laying undisturbed (heated water: 513 ± 10.5, natural water: 470 ± 7.3). Eggs agitated during incubation hatched with greater synchrony than those incubated undisturbed. Larvae incubated at river temperatures were larger than those incubated in heated water. Our findings revealed that eggs can hatch at different incubation stages during the ontogeny when exposed to varying environmental stimuli.

Les auteurs tentent de déterminer si les oeufs de lavaret du Bourget (*Coregonus lavaretus*) soumis à une agitation induite ont une plus courte période d'incubation avant l'éclosion que des oeufs en incubation non perturbée. Ils ont divisé les oeufs oeillés en quatre groupes dont deux ont été incubés aux températures du milieu fluvial et les deux autres, dans de l'eau chauffée. La température du milieu fluvial s'élevait à 2°C au début de l'expérience et est passée à plus de 10°C après l'éclosion tandis que la température de l'eau chauffée a varié de 6,5 à 8,5°C. Un échantillon à quatre répétitions incubé à chaque température a été continuellement agité avec de l'eau courante tandis que deux échantillons à quatre répétitions chacun ont été incubés sans être dérangés. Aux deux températures d'incubation, les oeufs gardés en mouvement par l'eau courante ont éclos en moins de degrés-jours (eau chauffée : 380 ± 6,4; eau naturelle : 417 ± 6,6) de la fertilisation à l'éclosion à 50 % que ceux gardés en milieu non perturbé (eau chauffée : 513 ± 10,5; eau naturelle : 470 ± 7,3). Les oeufs agités pendant l'incubation ont éclos en plus grande synchronisation que ceux incubés en milieu non perturbé. Les larves incubées aux températures du milieu fluvial étaient plus grosses que ceux incubées dans l'eau chauffée. Les résultats de l'expérience révèlent que les oeufs peuvent éclore à différents stades d'incubation pendant l'ontogénèse quand ils sont exposés à divers stimuli environnementaux.

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During a 3-yr study, Næsje et al. (1986) found a significant, positive correlation between increasing water discharge and the number of newly hatched whitefish (*Coregonus lavaretus*) and cisco (*C. albula*) larvae per unit volume of water samples that passively drifted out of a known river spawning ground. These authors proposed that a possible reason for this correlation was that increased egg movements due to increased water current induced hatching. Several authors (e.g. John and Hasler 1956; Bidgood 1974; Luczynski 1984a) have previously indicated that external stress exerted on coregonid eggs may result in a reduced egg incubation period and earlier hatching. However, none of these investigators presented supportive data. In addition to mechanical disturbance, rise in water temperature (Lindroth 1957; Luczynski and Kolman 1985; Luczynski 1985), low oxygen levels (Luczynski 1984a), and amount of heat (degree-days) (Colby and Brooke 1973; Brooke 1975; Luczynski and Kirklewska 1984) are environmental stimuli that induce hatching of coregonid eggs. According to Yamagami (1970) and Hagenmaier (1974), these environmental stimuli induce hatching by influencing the secretion of hatching enzymes, chorionases.

In the present study, we have experimentally tested whether agitation of eggs with increased water movements affects the hatching time of whitefish (*C. lavaretus*) eggs.

Materials and Methods

At the Ims Hatchery, eggs from 21 female (total lengths 265–365 mm) and milt from five male (295–315 mm) whitefish caught in River Imsa were fertilized on December 11, 1985.

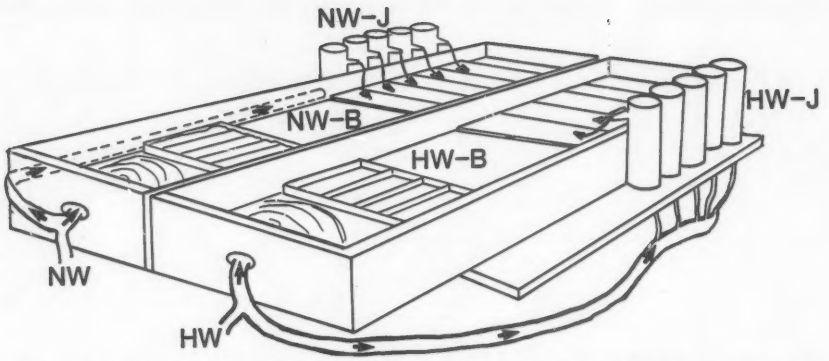

FIG. 1. Experimental apparatus. The incubation and receiving baskets had high walls to prevent mixing of hatched larvae. NW = natural water, HW = heated water, J = jars, and B = baskets.

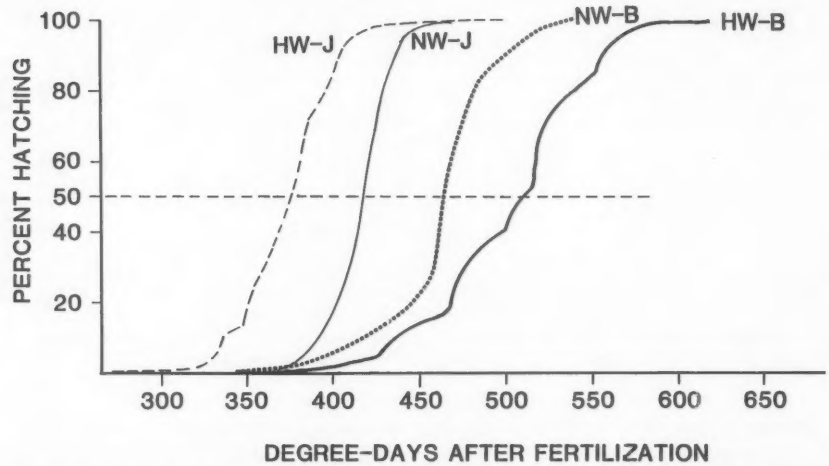

FIG. 2. Cumulative hatch of eggs incubated in jars (HW-J) and baskets (HW-B) in heated water and jars (NW-J) and baskets (NW-B) in natural water.

The eggs were divided into 20 groups of about the same size. All groups were incubated in unheated river water laying still in baskets for 140 degree-days (number of days multiplied with mean daily water temperature from base 0°C) until February 11, 1986. The groups were incubated until the eyed egg stage at the same temperature regimes varying from 4.0 to 1.9°C. Then, 10 groups were put into heated water (HW) (incubation temperatures from 6.5 to 8.5°C) and 10 groups were left in unheated River Imsa water (NW) (incubation temperatures varied from 2.2 to 11.1°C). Five groups incubated under each temperature regime were put into glass jars (HW-J and NW-J) whereas the other 10 groups were incubated in baskets (HW-B and NW-B). Eggs incubated in jars were continuously stirred by moving water injected at the base of each jar, while those in the baskets were still (Fig. 1). The water flowthrough was the same in all jars, 1.0–1.3 $L \cdot min^{-1}$. The depth of eggs in the baskets did not exceed two layers. The baskets floated in two wide troughs with a water flowthrough of 4.0 $L \cdot min^{-1}$ in the troughs (Fig. 1).

The oxygen content of the water in both jars and near the eggs in the baskets approximated 100% saturation. Fungus was controlled by treatment with malachite green weekly until the eyed egg stage and once with iodine at the eyed egg stage. Dead and fungus-infected eggs were carefully removed once weekly until the eyed egg stage and daily thereafter.

The incandescent light in the hatchery was turned on at 0800 and off at 1600. Each day at 0800 and 1500, all hatched embryos were collected and counted and water temperatures were measured. The total number of eggs in each of the five replicates varied from 610 to 1228 in HW-J, 414 to 649 in HW-B, 324 to 1189 in NW-J, and 461 to 1037 in HW-B.

Total lengths of 10–30 newly hatched larvae were measured daily (0.1 mm) for each of the four groups (HW-J, HW-B, NW-J, and NW-B) from the day of 2% cumulative hatching (defined as day 1 of the hatching period in Fig. 4). Relative sizes of yolk sacs of early-sampled (first 10 d of hatching period) and late-sampled (last 5 d of hatching period) hatching larvae were compared by measuring fish depths (0.1 mm). Fish depths were measured from the dorsal to the ventral side at the position of the yolk sac (see Fig. 4).

Results

Eggs kept in motion by water in the jars (HW-J and NW-J) required fewer degree-days (base 0°C) from fertilization to

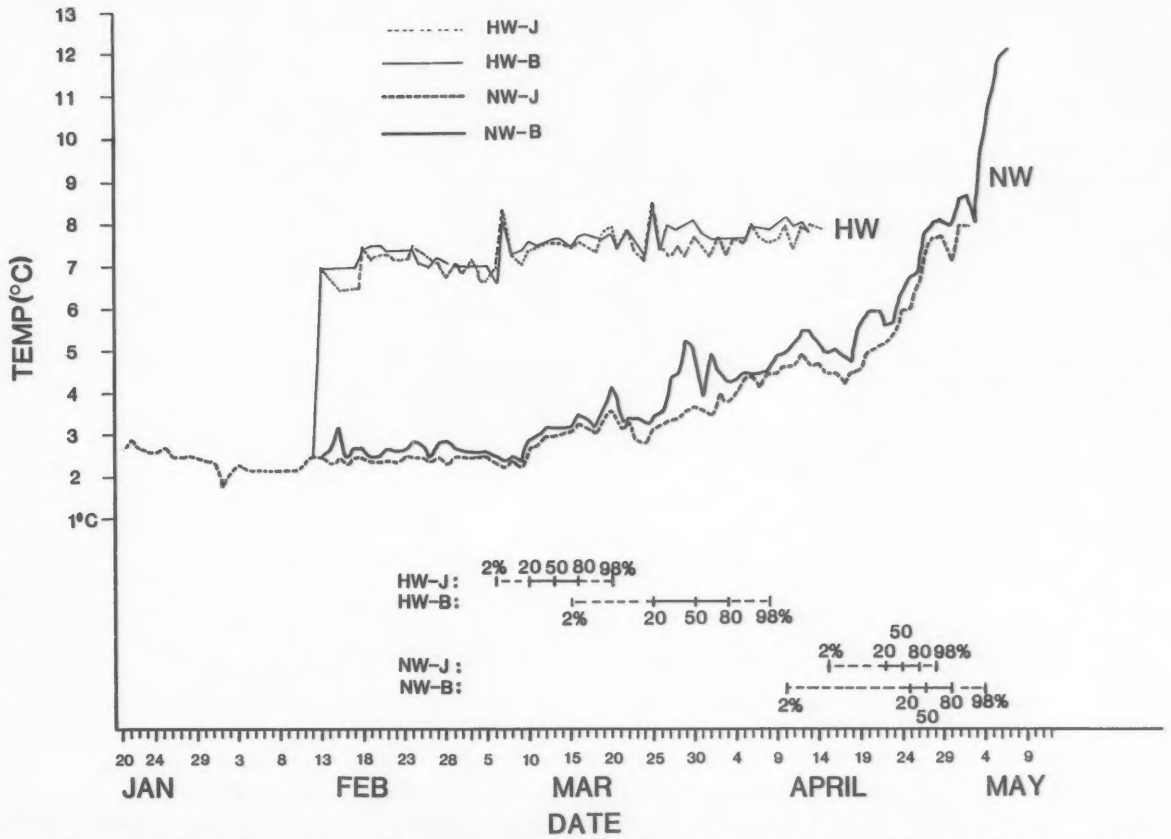

FIG. 3. Mean daily temperatures (0800 and 1500) and percent cumulative hatching of eggs in jars (J) and baskets (B) incubated in heated water (HW) and natural river water (NW).

hatching than those incubated in baskets (HW-B and NW-B) (Fig. 2). The number of degree-days from fertilization to 50% hatching (±SD) for HW-J and HW-B was 380 ± 6.4 and 513 ± 10.5 (t = 24.2, 8 df, $P < 0.0001$), respectively, whereas that for NW-J and NW-B was 417 ± 6.6 and 470 ± 7.3 (t = 12.0, 8 df, $P < 0.0001$), respectively. The difference in incubation time between jar and basket groups was larger in heated water (380–513) than in natural River Imsa water (t = 9.61, 8 df, $P < 0.0001$). Temperatures of the heated water fluctuated only slightly during incubation whereas the temperature of the natural River Imsa water increased toward the end of the incubation period (Fig. 3).

Eggs agitated in jars hatched in narrower synchrony than those incubated in baskets (t = 9.94, 18 df, $P < 0.0001$) (Fig. 3). Hatching periods (2–98%) of NW-J, HW-J, NW-B, and HW-B lasted for 12 ± 3, 14 ± 0.9, 23 ± 3, and 24 ± 2 d, respectively. There were no significant differences in hatching time either between the five replicates incubated in jars under two water temperatures or in the five replicates incubated in baskets under two water temperatures ($P > 0.05$).

The number of eggs that hatched per time unit in light and dark periods did not differ significantly (Wilcoxon's signed-rank test, $P > 0.05$), except for eggs incubated in jars with heated water. In the latter case the hatching rate was highest in light periods (Z = 3.0, $P < 0.01$).

Larvae incubated in natural water temperatures were longer than those incubated in heated water (NW-J versus HW-J: t = 12.92, 128 df, $P < 0.0001$; NW-B versus HW-B: t = 5.25, 121 df, $P < 0.0001$). Mean lengths of NW-J, NW-B, HW-J, and HW-B at 50% cumulative hatching were 13.7 ± 0.71 (N = 70), 13.3 ± 0.70 (N = 59), 12.0 ± 0.72 (N = 60), and 12.6 ± 0.71 mm (N = 64), respectively. Furthermore, larval length increased significantly during the hatching periods (t-tests, all $P < 0.0001$) (Fig. 4), and a significant correlation between total length and time of hatching was found in all groups (R^2_{NW-J} = 0.90, R^2_{NW-B} = 0.86, R^2_{HW-B} = 0.45, and R^2_{HW-J} = 0.91).

The yolk sac decreased significantly in size (Y, millimetres) during the hatching period for the two groups incubated in jars (HW-J: early hatching Y = 2.0 ± 0.23 (N = 22), late hatching Y = 1.7 ± 0.31 (N = 30), t = 3.20, 50 df, $P < 0.01$; NW-J: early hatching Y = 2.4 ± 0.26 (N = 38), late hatching Y = 1.9 ± 0.26 (N = 33), t = 6.95, 69 df, $P < 0.0001$). For the two groups incubated in baskets, there was no significant decrease in the size of the yolk sac during the hatching period ($P > 0.05$).

Discussion

Induced agitation of whitefish eggs caused by water movements reduced incubation time. This finding supports the

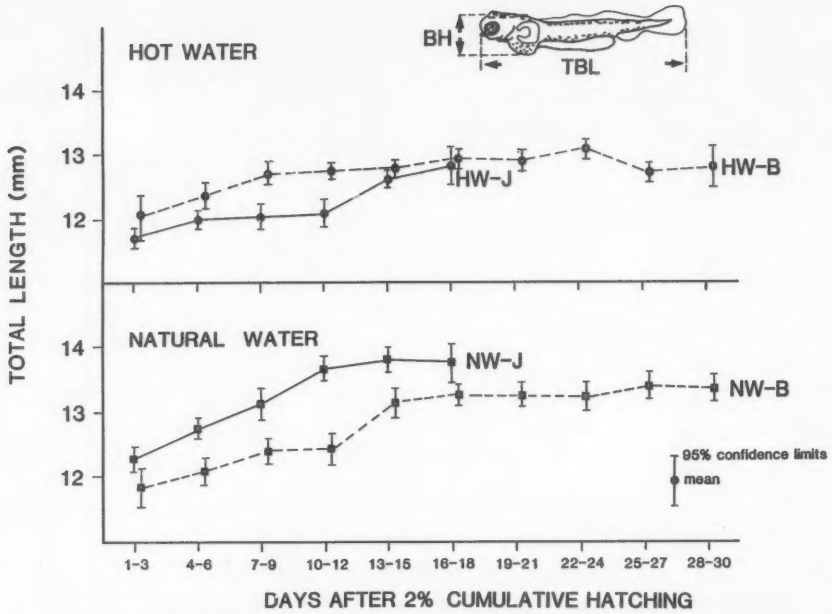

FIG. 4. Embryo length at hatching. Cumulative hatching time is defined as the period between 2 and 98% hatch. Maximum depth (BH) at the position of the yolk sac and total length measurement (TBL) locations are indicated. Replicates of eggs incubated in baskets in natural water (NW-B), eggs incubated in jars in natural water (NW-J), eggs incubated in baskets in heated water (HW-B), and eggs incubated in jars in heated water (HW-J) are presented.

hypothesis of Næsje et al. (1986), who suggested that egg movements caused by increased water flow during spring freshets induced hatching of coregonid eggs incubated in rivers. The present results differ from those of Colby and Brooke (1973) who did not find any significant difference in incubation time of lake herring (*Coregonus artedii*) eggs incubated when lying still and when slightly moved by increased water flow. Hamor and Garside (1976), on the other hand, showed that agitation of Atlantic salmon (*Salmo salar*) eggs caused by high water exchange rate has its prime importance during the hatching process. They suggested that agitation results in more immediate demands of locomotory activity which contributes to the rupture of the zona radiata.

Hatching of whitefish eggs results from a combination of movement of the embryo within egg shells and the action of hatching enzymes, chorionases (Price 1940; Smith 1957). Our results indicate that not only larval activity within the shell but also movement of the eggs induces hatching. When working with medaka (*Oryzias latipes*), Yamagami (1981) hypothesized that mechanical stimuli could cause secretion of hatching enzymes by the gland cells. Our studies with coregonids (Næsje et al. 1986; this study) indicate that one such stimulus is the agitation of eggs by water movements.

The lengths of the hatching periods of agitated eggs were significantly shorter than the hatching period of unagitated eggs. This indicates that the agitation may increase the secretion of the hatching enzymes or increase the activity of the secreted enzymes.

Choriolysis is temperature dependent (Gray 1926; Hayes 1942; Luczynski 1984a, 1985), e.g. the optimum temperature for the activity of hatching enzymes in rainbow trout (*Salmo gairdneri*) is 18°C (Hagenmaier 1974). The temperature dependence of the choriolysis may explain the greater difference in incubation time (degree-days) between stressed and unstressed eggs in heated water than between those in unheated water in the present study. In heated water, temperature was always high enough to allow early choriolytic activity in the stressed group. In unheated water the situation was different. Eggs from stressed groups did not start hatching before water temperature was 3–4°C. At lower temperatures, the hatching enzymes have low activity. Several authors (Luczynski 1984a, 1984b; Luczynski and Kolman 1985; Kolman and Luczynski 1986) have delayed hatching of coregonid eggs by keeping them in cold water (1–2°C). They claimed that secretion of choriolytic enzymes is very low, or nonexistent, at temperatures below 3°C, or that choriolytic activity itself is very low at such low temperatures. However, hatching of coregonid eggs may occur at lower temperatures. Bidgood (1974) reported hatching of eggs of lake whitefish (*Coregonus clupeaformis*) when the incubating water temperature was 2°C continuously from fertilization. Furthermore, in River Gudbrandsdalslågen, hatching of whitefish and cisco eggs starts at temperatures below 1°C.

Early-hatching eggs yielded smaller larvae than those hatching late. This finding is supported by Bidgood (1974) and Brooke (1975), who found that at any given temperature, the first hatching fry of *C. clupeaformis* were shorter than those hatching later. In chinook salmon (*Oncorhynchus tshawytscha*), on the other hand, alevin lengths increased to a maximum during the hatching period. Larvae hatching later did not decrease significantly in length (Heming 1982), corresponding with our results. Larvae hatched at low water temperatures were larger than those incubated in warmer water, supporting earlier find-

ings by Price (1940), Colby and Brooke (1970), Brooke (1975), and Luczynski et al. (1986) who also supported that coregonids incubated in cold water were larger than those incubated in warm water.

Agitation of eggs led to more synchronous hatching, which agrees with Bidgood (1974) who found that eggs rolling in jar cultures had a shorter hatching period that those incubated while lying still in trays under the same temperature regime. This also agrees with field data presented by Næsje et al. (1986) in River Gudbrandsdalslågen when the duration of the hatching period was negatively correlated with the size of the spring freshet.

This study demonstrates that agitation induces earlier hatching of whitefish eggs. If no specific stimulus is given, hatching will be delayed. This connection between environmental stress and hatching demonstrated in the present study may occur among other fish species. Few studies on this aspect of recruitment have been published.

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Relative Contributions of Dietary and Waterborne Zinc in the Rainbow Trout, *Salmo gairdneri*

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Rainbow trout, *Salmo gairdneri*, were fed purified diets with zinc concentrations ranging from deficient to excessive (1, 90, 590 μg Zn·g^{-1}) and simultaneously exposed to a range of waterborne [Zn] (7, 39, 148, 529 μg Zn·L^{-1}). After 1 wk, fish fed the deficient diet, at ambient waterborne [Zn], had low plasma [Zn] which decreased further during the 16-wk experiment. Growth ceased after 12 wk; hematocrit and plasma protein were depressed. Both whole body [Zn] and body burden decreased by 16 wk, but most other elements were elevated. Increasing waterborne [Zn] alone increased plasma [Zn], whole body [Zn], and growth in a graded manner and normalized hematocrit, plasma protein, and other whole body elements. Increasing dietary [Zn] to 90 μg Zn·g^{-1} at ambient waterborne [Zn] prevented depression of plasma [Zn] and permitted normal growth and whole body [Zn]. Zinc uptake from water, probably across the gills, was independent of uptake from the diet since at any dietary [Zn], increasing the waterborne [Zn] resulted in increased whole body [Zn]. Even when dietary [Zn] was adequate, the waterborne contribution was as high as 57%, and 100% when the dietary [Zn] was deficient. There were no toxic effects on any of the variables measured.

Des truites arc-en-ciel, *Salmo gairdneri*, ont été soumises à un régime alimentaire purifié dont les concentratrions en zinc variaient d'insuffisantes à excessives (1, 90, 590 μg·g^{-1} de Zn) et simultanément exposées à des milieux (eau) de différentes [Zn] (7, 39, 148, 529 μg·L^{-1} de Zn). Au bout d'une semaine, la [Zn] plasmatique des poissons soumis au régime carencé et exposés à la [Zn] ambiante était faible et a continué à diminuer pendant les 16 sem de l'expérience. La croissance s'est arrêtée après 12 sem; l'hématocrite et le taux de protéine dans le plasma avaient diminué. La [Zn] corporelle et la charge corporelle en Zn ont diminué au cours des 16 semaines, mais celles de la plupart des autres éléments avaient augmenté. Le seul fait d'augmenter la [Zn] dans l'eau a entraîné une hausse graduelle de la [Zn] plasmatique, de la [Zn] corporelle et de la croissance ainsi que la normalisation de l'hématocrite, du taux de protéine dans le plasma et des concentrations des autres éléments corporels. L'augmentation de la [Zn] de la ration alimentaire à 90 μg·g^{-1} de Zn pour les poissons exposés à la [Zn] ambiante a permis d'éviter la baisse de la [Zn] plasmatique et d'obtenir une croissance et une [Zn] corporelle normales. La captation du zinc provenant du milieu, probablement à travers les branchies, était indépendante de la captation du zing présent dans l'alimentation, en effet, peu importe quelle était la [Zn] dans l'alimention de la [Zn] de l'eau se traduisait par une augmentation de la [Zn] corporelle. Même lorsque la [Zn] dans l'alimentation était appropriée, la contribution du milieu pouvait atteindre 57 %; elle était de 100 % lorsque la [Zn] dans l'alimentation était insuffisante. Les variables mesurées n'ont pas permis de déceler d'effets toxiques.

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A dietary requirement for zinc has been documented in rainbow trout (*Salmo gairdneri*) based upon low growth, high mortality, cataracts, and fin erosion induced in trout fed a diet containing 1–4 μg Zn·g^{-1} (Ogino and Yang 1978; Wekell et al. 1983). Supplementing the diet to 15–30 μg·g^{-1} alleviated these symptoms. Diets containing high levels of calcium (and phosphate) and/or phytate (myoinositol hexaphosphate) increased the requirement for dietary zinc in freshwater salmonids, since they reduced zinc bioavailability (Ketola 1979; Hardy and Shearer 1985; Richardson et al. 1985). Poor growth, cataracts, and high mortality were reported, together with low plasma and whole body [Zn]. In contrast, high levels of dietary zinc appear fairly benign. Thus, Wekell et al. (1983) found no growth inhibition in fingerling trout over a dietary range of 440–1700 μg Zn·g^{-1}. Liver and whole blood [Zn] increased significantly over this range but in an attenuated fashion, whereas gill [Zn] increased almost linearly. No mortality or toxic symptoms occurred.

While dietary concentrations up to 1700 μg Zn·g^{-1} are well tolerated, waterborne concentrations of 1 μg Zn·mL^{-1} (i.e. 1000 μg ·L^{-1}) are well into the toxic range for rainbow trout (Spear 1981). Detrimental effects on ionoregulation, acid–base balance, and gas exchange have been documented (Skidmore 1970; Lewis and Lewis 1971; Sellers et al. 1975; Spry and Wood 1984, 1985). The maximum acceptable toxicant concentration based upon fry mortality for rainbow trout in hard water was 320–640 μg Zn·L^{-1} (Sinley et al. 1974). Even below this range, elevated waterborne [Zn] depressed growth in several

species due to appetite suppression and/or decreased conversion (Bengtsson 1974; Watson and McKeown 1976; Farmer et al. 1979).

Zinc can be accumulated directly from the water. Holcombe et al. (1979) exposed brook trout (*Salvelinus fontinalis*) for 24 wk to a waterborne [Zn] of 534 $\mu g \cdot L^{-1}$ and found threefold increases above controls in gill, kidney, and opercular bone [Zn], whereas brain and muscle were unaffected. Ration manipulation was used by Farmer et al. (1979) to study its effect upon zinc uptake from water. Freshwater-adapted Atlantic salmon (*Salmo salar*), fed a practical diet to satiation (dietary [Zn] unspecified), reached steady-state whole body concentrations after 50 d. These steady-state whole body [Zn] reflected waterborne [Zn] in a concentration-dependent fashion. Restriction of the ration to 2 or 3.5% body weight$\cdot d^{-1}$ resulted in continual accumulation over the 80-d exposure. The rate of accumulation was directly proportional to the waterborne [Zn].

Relative contributions of dietary versus waterborne zinc to zinc uptake have been assessed in marine fish. In plaice (*Pleuronectes platessa*), food was the major source for larvae and adults when waterborne [Zn] was low (15 $\mu g \cdot L^{-1}$), based upon ^{65}Zn accumulation (Pentreath 1973, 1976). However, in the same species, water contributed <10% at 100 $\mu g \cdot L^{-1}$ but increased to 50% at 600 $\mu g \cdot L^{-1}$ (Milner 1982), indicating that waterborne input can be significant. Willis and Sunda (1984) used a model food chain (*Chlamydomonas* → *Artemia* → fish) and a single waterborne [Zn] (0.21 μg free $Zn \cdot L^{-1}$) to estimate relative contributions in mosquito fish (*Gambusia affinis*) and spot (*Leiostomus xanthurus*). Food supplied 77% of the isotope load in mosquito fish after 120 d and 82% in spot after 30 d. Marine fish drink considerable amounts of the medium (~4–13% body weight$\cdot d^{-1}$) (Smith 1930; Shehadeh and Gordon 1969), and this could contribute half or more of the waterborne input. The remaining input from the water was suggested to be passive, being driven by the large gradient formed by the adsorption of zinc on the gill (Pentreath 1973).

There have been no directly comparable studies in freshwater, where drinking rates are known to be negligible (Shehadeh and Gordon 1969; Oduleye 1975). Stary et al. (1982) exposed guppies (*Lebistes reticulata*) to ^{65}Zn in either the water or in the diet. They concluded that diet was the more important factor. Pumpkinseed sunfish (*Lepomis gibbosus*), exposed to ^{65}Zn in either the diet or the water, accumulated ^{65}Zn faster from the diet, whether the diet was natural (snail) or purified (Merlini et al. 1976). In contrast, waterborne zinc was the main source for goldfish (*Carassius auratus*) (no details, Berg and Brazzelli, cited in Merlini et al. 1976).

It thus appears that in both freshwater and seawater fish there is a capacity to accumulate zinc through both routes, but the dietary source is usually the more important. However, the interactions between the two pathways appear highly complex and dependent upon the zinc concentrations in the two sources. It remains unclear whether the waterbourne route can replace the dietary route.

The aims of the present experiment were to (1) see if a dietary [Zn] deficiency similar to that previously reported could be induced in juvenile rainbow trout, and if so, whether trout could obtain sufficient zinc from the water to overcome this deficiency, (2) quantify uptake through the two pathways and to see if uptake through one route influenced uptake through the other, (3) look for toxic effects of higher dietary or waterborne zinc, and (4) see if metabolism of other elements (e.g. calcium) was altered by either dietary and/or waterborne zinc.

Materials and Methods

Experimental Design

Rainbow trout (*Salmo gairdneri*) fingerlings (3–4 g) were purchased from Spring Valley Trout Farm, Petersburg, Ont. They were held in flowing Burlington tap water dechlorinated by carbon filtration and sulphite addition. The photoperiod was 18 h light : 6 h dark, and ambient [Zn] was 7 ± 8 $\mu g \cdot L^{-1}$ (mean ± SD). Fish were held for 2 wk in a large holding tank and gradually changed from a commerical diet (~200 μg $Zn \cdot g^{-1}$, Martin Feed Mills, Elmira, Ont.) to the semipurified diet described below, and the temperature was raised from 11 to 15°C. After this initial acclimation, fish were transferred to the experimental battery which consisted of a grid of 30 tanks. Each tank was approximately a 30-cm cube holding 20–23 L of water. Water flowed continuously to the tanks from mixing chambers at 300 $mL \cdot min^{-1} \cdot tank^{-1}$, giving a 95% particle replacement time of 3–4 h (Sprague 1969). Tanks were gently aerated to ensure >90% saturation.

The experimental design was a complete block factorial consisting of 3 waterborne [Zn] levels (ambient plus 2 treatments) × 3 dietary [Zn] levels × 3 replicates (blocks) for a total of 27 tanks. To determine the effect of an even greater waterborne [Zn] on the experimental variables, particularly whether or not toxicity would occur, the remaining three tanks were used for three replicates of a further increase in the waterborne [Zn] to 529 $\mu g \cdot L^{-1}$ at the highest dietary [Zn] (590 $\mu g \cdot g^{-1}$). The experiment ran for 16 wk. The waterborne zinc (as $ZnSO_4 \cdot 7H_2O$) was metered by a peristaltic pump (Gilson minipulse) into the mixing cells of the diluter from three stock bottles. Daily measurements of waterborne [Zn] gave means ± SD of 7 ± 8 $\mu g \cdot L^{-1}$ for ambient and 39 ± 14, 148 ± 35, and 529 ± 85 $\mu g \cdot L^{-1}$ for the three elevated levels. Temperature was 15.3 ± 1.3°C. Weekly measurements of pH, dissolved oxygen, and conductivity were 8.16 ± 0.23, 8.8 ± 1.0 $mg \cdot L^{-1}$, and 281 ± 10 μS. Previously reported hardness and alkalinity values (as $CaCO_3$) were 135 ± 2 and 90 ± 4 $mg \cdot L^{-1}$ (Hodson et al. 1978).

On the basis of past experiments under the same conditions, some aggression and subsequent mortality was expected (Hodson et al. 1978). Small lengths of 1-in. inside diameter PVC pipe were added to provide shelter, and the tank lids were covered with black plastic on week 2 in an attempt to minimize this effect, but aggression persisted. Typically, fish showing severe signs of aggression did not feed. Opercles and fins became eroded until the fish was unable to propel itself or remain upright, and fungal infestation was common. Fish that rolled over were considered moribund and were recorded and sacrificed. The same criteria were used for removal of diseased fish (see Results).

Diet

The semipurified diet formulated by Dr. J. W. Hilton (University of Guelph) (Table 1) was calculated to provide all essential nutrients. Dietary [Zn] was nominally 0, 100 (practical level), and 700 μg $Zn \cdot g^{-1}$ (as sulphate). This was confirmed by dry ashing at 400°C and atomic absorption spectophotometry, and independently by neutron activation analysis, yielding measured [Zn] = 1, 90, and 590 μg $Zn \cdot g^{-1}$. The proximate composition was 40% protein, 15% lipid, 8% ash, and 9% moisture. All diets were steam-pelleted and crumbled to sizes readily acceptable to the fish over the course of the experiment.

TABLE 1. Formulation of basal diet fed to rainbow trout. The mineral premix provided the following (g·kg dry diet^{-1}): $CaHPO_4 \cdot 2H_2O$, 30; $CaCO_3$, 3; NaCl 15; K_2SO_4, 20; $MgSO_4$, 10; $FeSO_4 \cdot 7H_20$, 0.7; $MnSO_4 \cdot H_2O$, 0.3; $CuSO_4 \cdot 5H_2O$, 0.16; KI, 0.015. The vitamin premix provided the following (mg·kg dry diet^{-1}, unless noted otherwise): thiamine, 10; riboflavin, 10; pantothenic acid, 10; niacin, 20; pyridoxine, 40; biotin, 0.5; folic acid, 20; vitamin B-12, 0.2; inositol, 500; ascorbic acid, 1000; choline chloride, 5500; retinyl palmitate, 7000 iu; cholecalciferol, 3000 iu; DL-α-tocopheryl acetate, 200 iu; menadione, 50; butylated hydroxytoluene (BHT), 25.

Ingredient	Concentration (%)
Egg albumin	40
Gelatin	10
Alpha-starch	10
Cerelose	10
Cellulose	5
Vitamin premix	2
Mineral premix	8
Fish oil	15

Fish were conditioned to the 90 μg Zn·g^{-1} diet for 1 wk prior to their placement in the test battery.

To begin the experiment, the rainbow trout fingerlings were added to the tanks in a stratified hierarchical fashion, 34 per tank. Small adjustments were necessary in a few tanks to standardize the initial starting weights. Trout were fed to satiation (cessation of feeding) three to four times daily (except prior to sampling, see below), decreasing to two to three times daily after 6 wk. Food consumption was monitored by feeding fish from preweighed aliquots of the diet. All diets were stored at −20°C when not in use. Fish weight was measured by batch weighing (netting all fish in the tank into a tared, water-filled bucket) once a week for the first 4 wk and then every other week for the remainder of the experiment. When weighing coincided with sampling, the sample was taken first and then the remaining fish in the tank were weighed and the weight of fish sampled added to the tank weight. Mean fish weight was calculated as the total fish weight divided by the number of fish per tank.

Sampling

For each sample day (weeks 0, 1, 2, 4, 8, and 16), 90 fish were sampled in random order, three from each tank. Food was withheld for 36–48 h prior to sampling. Fish were netted indiscriminately from the tank and blood samples collected in ammonium heparinized glass hematocrit tubes from the severed caudal peduncle. Hematocrit tubes were stored briefly on ice and then centrifuged. Hematocrit was read and plasma recovered from the tubes for later analysis for Na, Ca, Mg, and Zn (all by atomic absorption spectrophotometry on a Varian AA-1275) and, for week 16, total protein determined by refractometry on an American Optical TS meter (Alexander and Bell 1980). Fish weight and standard length were recorded and the general appearance of the fish noted for overt pathology, particularly cataracts and fin erosion, as these are symptomatic of zinc deficiency in trout (Ogino and Yang 1978). Fish were individually bagged and frozen at −20°C for whole body ion analysis.

Neutron Activation Analysis

Whole body element concentrations were determined by exposure of fish samples to thermal neutrons in the McMaster University reactor. Fish from the 16-wk sample were slit longitudinally to expose the gut and then freeze dried to determine water content. After the intestines were examined, and any food or fecal matter removed, the entire fish was sealed in 7-g polyethylene vials. For short-lived isotopes (I, Br, Mg, Cu, Na, V, K, Cl, Mn, Ca), the sample was irradiated in a neutron flux of 5×10^{12} n·cm^{-2}·s^{-1} for a 10-s irradiation. After a delay time of 120 s the samples were counted for 600 s using an APTEC hyperpure germanium detector (22% efficient with a 1.9 keV resolution at the 1332-keV ^{60}Co peak). Counts were accumulated on a Canberra multi channel analyzer model 40 or 90 equipped with internal live time correction and pile up rejection unit. Citrus leaf (NBS 1572) was used as the standard. For long-lived isotopes (Fe, Zn), the samples were loaded directly into the core (flux intensity 7.5×10^{12} n·cm^{-2}·s^{-1}) for 5–6 h. After a delay time of about 3 wk during which time the short- and medium-lived isotopes decayed, the samples were counted for a period of 900 s. Here the standard used was lobster hepatopancreas (TORT-1, National Research Council of Canada, Ottawa, Ont.). Different sample groups were standardized for variations in flux intensity by the use of flux wires.

Calculations and Statistics

Since the number of fish in each tank decreased due to sampling, mortality was expressed as a function of the total number of fish days accumulated per tank. Feed efficiency was the wet weight gain divided by the wet weight of feed given per tank. Feed intake (percentage of body weight per day) was calculated from the wet weight of feed given over the interval between fish weighings, the average of the weights at the two times, and the elapsed time. Condition factor was calculated as the wet weight (grams)/standard length^3 (centimetres) × 100. The apparent zinc retention (percentage) was calculated from the difference between the initial (week 0) and final (week 16) body burden (micrograms of zinc per fish) divided by the amount of zinc fed (dietary [Zn] × feed given), and expressed as a percentage. Estimates of the relative contribution of waterborne zinc to the final body zinc burden were arrived at by assuming that at ambient waterborne [Zn], uptake from the water was negligible, and that the entire zinc load was from the diet. For each water level above ambient, the difference between the initial and final body burden (micrograms of zinc per fish) *minus* the same difference at ambient [Zn] for that dietary level was considered the absolute amount contributed by the water. In turn, this was expressed as a percentage of the total accumulated burden over the 16 wk for that dietary treatment.

The balanced part of the experiment (i.e. all the treatments exclusive of the high water × high diet) was analysed by two-way ANOVA having 3 dietary × 3 waterborne × 3 replicates, with 3 observations per cell for a total of 81 observations. Interaction terms between replicates and the two other factors were significant in only two instances. In all other cases, therefore, the pooled interaction variance was used to test the main effects. Where there were significant treatment effects, individual means were tested for significant differences by Peritz' *F* test (Harper 1984).

Results

Growth and Survival

Although fish were sampled at weeks 0, 1, 2, 4, 8, and 16, the trends shown over the course of the experiment were borne out by the results at week 16. Thus, except in rare instances,

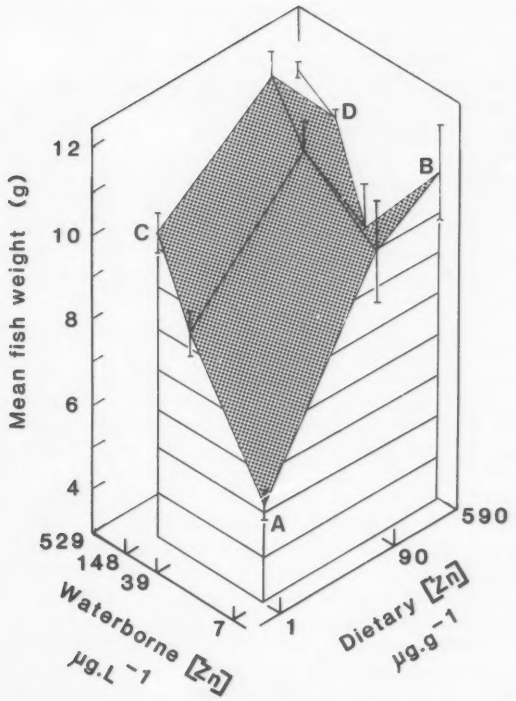

FIG. 1. Mean fish weight at the end of the experiment as a function of waterborne and dietary inputs. Values are means ± SEM (9), (3 fish from 3 different replicates). Letters ABCD are simply for orientation; see text for details.

only the week 0 (hereafter called the initial values) and week 16 values will be discussed.

The fish fed actively, but growth was less than on practical diets, or even casein-based semipurified diets (e.g. Hodson et al. 1980). Aggression may have been a factor here. There was no significant growth over the first 4 wk and no difference in growth among treatments over the first 6 wk. Thereafter, a gradual separation occurred, most clearly seen in the fish on the lowest dietary [Zn] (1 $\mu g \cdot g^{-1}$) at the lowest waterborne [Zn] (7 $\mu g \cdot L^{-1}$). These fish (hereafter called the deficient fish) had noticeably slower growth by week 8. The deficient fish continued to grow until week 12, when the mean weight per tank had risen from 3.20 ± 0.03 (3) to 5.49 ± 0.28 (3) g. No further growth occurred. An increase in zinc either from the diet alone (Fig. 1, edge AB) or from the water alone (Fig. 1, edge AC) provided for continued growth in a concentration-dependent fashion. By the end of the experiment, fish at the lowest treatment combination were less than half the size of the fish in the other treatments (Fig. 1), illustrating significant effects of both the water and dietary treatments. Based upon growth and mortality, there were no toxic effects of the high levels of either dietary or waterborne [Zn]. In fact, fish vigour appeared to increase with increasing waterborne [Zn].

Fish were fed about 2.5–4.0% of their body weight·d^{-1}. Intake tended to be lower at the highest dietary [Zn] (590 $\mu g \cdot g^{-1}$), perhaps reflecting a palatability effect. Over the last 2 wk of the experiment, deficient fish were fed more than any other treatment, and although the feed efficiency values were low for all treatments (range of means 19 ± 5 to 41 ± 9% (3)), fish in the deficient diet had an efficiency of zero. Despite the fact that fish in some treatments did not grow after 12 wk, there was no significant difference in condition factor among treatments (1.30 ± 0.04(9) to 1.35 ± 0.03 (9)), an effect that may have been masked by an apparently, but not significantly, higher water percentage in the deficient treatment (73.4 ± 0.5%) compared with the other treatments (means ranging from 70.0 ± 0.07 to 72.1 ± 0.6%).

Some disease and mortality occurred. Mortality from other than accidental causes was 7% (73/1020). Of this, 49% was due to nodular gill disease (Daoust and Ferguson 1985), aggression (26%), indeterminate causes (18%), and fungal infestation (7%). Disease mortality persisted despite formalin treatments (5-min immersions in 1:8000 dilution) in weeks 8 and 10. ANOVA indicated that disease was treatment related. There was a highly significant decrease in mortality with increasing waterborne [Zn]. Zinc-deficient fish seemed particularly susceptible to both nodular gill disease (most prevalent) and fungal infestation. There were only two incidents of gross cataracts, both in zinc-deficient fish. At the end of the experiment, all fish were examined for the presence or absence of nodules on the gill. There was clear evidence of the disease at all dietary treatments in fish at the lowest (i.e. ambient) waterborne [Zn]. At increased waterborne [Zn], however, very little evidence of the disease was found.

Blood Variables

Hematocrits at week 16 (ranging from 30.2 ± 1.3 (9) to 36.1 ± 2.0% (9)) were similar to the week 0 value, except for the fish in the deficient treatment which were slightly anemic (25.9 ± 2.1% (9)) due to a significant diet effect. Plasma protein was also depressed in the deficient treatment (2.1 ± 1.0 $g \cdot 100\ mL^{-1}$ (9) compared with all other treatments (3.4 ± 0.7 (9) to 4.0 ± 0.8 (9)). There was a significant diet effect and a water–diet interaction. Plasma concentrations of major ions did not change over the course of the experiment. Final values (millimoles per litre) were as follows: Na, 148 ± 2 to 158 ± 2; Ca, 2.2 ± 0.3 to 2.6 ± 0.1; Mg, 1.0 ± 0.1 to 1.1 ± 0.1 (9).

In contrast, the effect of treatment upon plasma [Zn] was striking. There were significant water and diet effects, but no interaction. Initial values were 0.15 ± 0.004 $mmol \cdot L^{-1}$ (88) (9.57 ± 0.23 $mg \cdot L^{-1}$). After only 1 wk of treatment, all of the fish on the deficient diet (Fig. 2A, edge AC) had significantly lower plasma [Zn]. Increasing the waterborne [Zn] increased the plasma [Zn], but still not to initial levels. Those on the intermediate diet were not different from initial values regardless of the waterborne concentrations. Fish on the high [Zn] diet were no different from the initial values at ambient waterborne concentrations, but as waterborne [Zn] increased, so did the plasma [Zn]. The lack of an interaction term between the diet and waterborne [Zn] confirmed that uptake via one route was independent of uptake via the other. The results at week 16 (Fig. 2B) were similar, except that at ambient waterborne [Zn] the plasma [Zn] from fish on the deficient diet had fallen to the point where it was scarcely detectable. Again, increases in waterborne [Zn] did permit some increases in plasma [Zn], but still not to initial levels. At higher dietary [Zn], there was generally a plateau around initial levels. Only at the highest waterborne and dietary level was there a significant increase above this plateau. Assuming the normal condition to be the medium diet and the ambient waterborne exposure, a decrement in dietary [Zn] from 90 to 1 $\mu g\ Zn \cdot L^{-1}$ under these water exposure conditions had a drastic effect on the plasma zinc status of

the fish. Increasing the dietary [Zn] from 90 to 590 μg Zn·L^{-1} caused no increase in plasma [Zn] (Fig. 2A, 2B, edge EB). Increasing the waterborne [Zn] from 7 to 148 μg Zn·L^{-1} had no effect on plasma [Zn] at higher dietary [Zn] (Fig. 2A, 2B, edge BD). This is indicative of plasma homeostatis over a broad range of dietary and waterborne [Zn].

Whole Body Elemental Analysis

Most of the whole body elements which were readily detectable using neutron activation analysis (Tables 2, 3) showed significant treatment effects. All the results are expressed on a dry weight basis. Since percent water was not significantly different across treatments, expressing results on a wet weight basis gave identical trends.

High waterborne [Zn] exposures had no effect on whole body elemental composition (other than zinc), further supporting the lack of any toxic effect on zinc on ion regulation, as seen previously with the plasma data. With the exception of whole body [Zn] (which showed a graded response) and [Cu] (which was unaffected), treatment differences were restricted to the deficient fish, and all showed virtually the same pattern of response to treatment, but with some variation in statistical significance.

Whole body [Ca] (Table 2) showed the treatment response most clearly. Elemental concentrations rose significantly, but only in the deficient fish. In contrast, the other treatments constituted a plateau which showed neither trends nor significant differences. This single cell caused significant treatment effects not only for dietary and waterborne [Zn] effects, but for the interaction as well. Similar significant responses were seen for Na, Mg, Br, and Fe. The elements V, K, and Cl showed similar elevations in deficient fish, but these were not significant. Whole body [Mn] was slightly different because in addition to elevations in whole body concentration seen in deficient fish, only the diet had a highly significant effect showing a decreasing whole body concentration as dietary [Zn] increased. Whole body [Cu] showed no treatment effects at all.

The generally higher elemental concentration in the deficient fish, compared with the other treatments at week 16, may have been an effect of fish size, since a scatter plot of whole body [Ca] versus fish weight irrespective of treatment (not shown) revealed an exponential decline with increasing size up to about 15 g. In fact, compared with initial values (Tables 2, 3), deficient fish had similar or lower concentrations for 8 of 11 elements. This again suggests a size effect. Only for Ca, Mn, and V were concentrations elevated significantly above initial values.

In contrast, whole body [Zn] (Fig. 3) reflected the loading to which the fish were subjected. Both diet and water were highly significant factors, and showed clear trends. There was no interaction between them, again indicating that zinc uptake by one route was independent of uptake via the other. The diet had by far the largest F value and was the major determinant of whole body [Zn] (Fig. 3). Fish on the deficient diet at ambient waterborne [Zn] (7 μg·L^{-1}) had the lowest whole body [Zn]. This did not increase when waterborne [Zn] was raised to 39 μg·L^{-1}, despite the fact that plasma [Zn] was higher (Fig. 2). Thus, plasma [Zn] under these conditions was more sensitive to loading than was whole body concentration. Only at a waterborne [Zn] = 148 μg Zn·L^{-1} was there an increased whole body concentration. At the other two dietary levels there were modest increases in the [Zn] as the waterborne [Zn] increased. Finally, at the highest dietary and the highest waterborne [Zn] there was a significant increase in whole body [Zn] to nearly double that of the week 0 value (week 0 = 1.41 ± 0.05 μmol·g^{-1} (15)). The deficient fish, in contrast, had whole body [Zn] of half the initial value. Unlike plasma [Zn] (Fig. 2B),

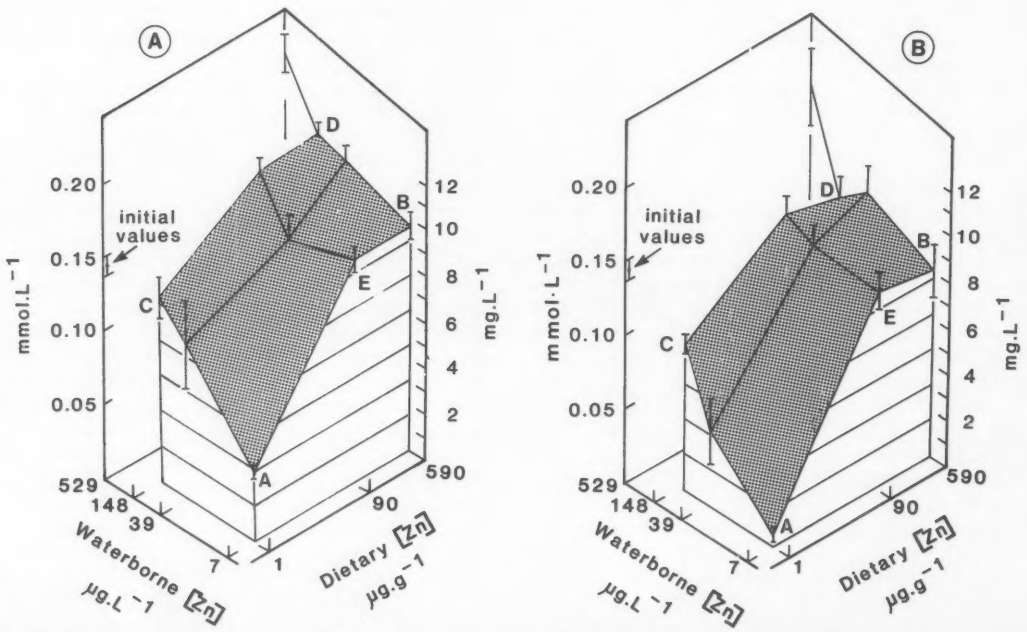

FIG. 2. Plasma [Zn] (mmol·L^{-1}) after (A) 1 wk of exposure and (B) 16 wk of exposure to various waterborne and dietary zinc input. Other details as in Fig. 1. Initial values (week 0) were 0.142 ± 0.004 mmol·L^{-1} (88).

TABLE 2. Whole body macronutrient (Martell 1975) concentrations at week 16. Values are μmol·g dry weight^{-1}, means ± SEM (9), except mean weight where units are g and n = 3.

		Weight (g)	Na	Mg	Cl	K	Ca
7	1	5.3±0.3	176±7	48±2	150±8	253±8	685±29
	90	9.9±1.3	140±9	40±2	127±10	225±9	458±25
	590	10.9±1.2	139±7	39±2	127±7	236±10	473±21
39	1	8.3±0.5	148±5	39±2	133±6	232±10	502±19
	90	11.3±0.7	147±8	40±2	137±10	238±10	465±28
	590	8.3±1.1	153±7	41±2	140±6	264±11	525±26
148	1	10.4±0.4	142±5	39±2	127±7	225±9	481±16
	90	12.6±0.6	134±6	38±2	126±6	222±10	423±19
	590	10.7±0.2	145±7	37±2	134±7	233±11	490±30
529	590	11.4±0.1	139±7	36±2	133±7	237±11	450±19
Initial values (n = 15, except weight, where n = 30)		3.2±0.0	180±5	59±3	145±5	337±7	462±11

TABLE 3. Whole body micronutrient (Martell 1975; Br is not classed as a nutrient) element concentrations at week 16. Values are nmol·g dry weight^{-1}, means ± SEM (9).

		Zn	Fe	Cu	Mn	V	Br
7	1	586±20	956±75	26±13	233±14	29±3	85±8
	90	1163±44	546±33	64±12	158±22	17±2	56±5
	590	1526±75	561±42	39±9	116±9	15±3	56±5
39	1	592±68	625±39	50±11	168±16	18±3	57±3
	90	1293±41	609±63	70±18	146±15	16±2	56±7
	590	1709±127	548±46	61±20	107±12	18±4	61±9
148	1	1050±121	555±32	42±7	153±24	18±3	56±2
	90	1398±44	540±16	52±9	135±14	12±2	50±8
	590	1840±96	665±103	39±11	141±13	20±2	57±4
529	590	2737±219	568±35	24±9	139±7	16±2	58±4
Initial values (n = 15)		1409±48	781±91	114±20	148±7	6±2	109±9

whole body [Zn] (Fig. 3) showed no clear plateau region where its level was independent of dietary and waterborne [Zn].

Discussion

Effects on Variables other than Zinc

Growth on the zinc-adequate diets was less than expected, even for albumin-based diets (Ogino and Yang 1978; Wekell et al. 1983). Reasons for this are unknown, despite the fact that the diet was formulated to meet all known requirements. One possibility is that since the albumin was not heat treated, avidin may have rendered biotin less available. Low growth notwithstanding, all plasma variables were in the reported range for the species (Hille 1982). As well, all 11 measured whole body element concentrations fell within the ranges given by Shearer (1984) for the corresponding fish size.

There were clearly no toxic effects of exposure to high dietary and/or waterborne [Zn] based upon growth, mortality, major plasma ions, hematocrit, or plasma protein. Similarly, Wekell et al (1983) reported that dietary [Zn] as high as 1700 μg·g^{-1} had no toxic effect on growth and mortality. We also found no effects of high [Zn] upon whole body element concentrations, despite indications that high dietary [Zn] interfered with accumulation of copper in the liver of rainbow trout (Knox et al. 1984), and waterborne [Zn] = 800 μg·L^{-1} blocked net calcium uptake in soft water (Spry and Wood 1985).

Dietary restriction of zinc (1 μg·g^{-1}), on the other hand, rendered trout zinc deficient, using the criteria of cessation of growth, significant mortality, and decreased plasma and whole body [Zn]. Decreased plasma protein concentration and low hematocrit in zinc-deficient fish have not previously been reported and may signify reduced protein synthetic ability. Cataract formation seen in earlier studies of zinc deficiency (Ogino and Yang 1978; Ketola 1979) occurred in only two zinc-deficient fish in the present study and was not seen in the study of Wekell et al. (1983) using a similar diet. Additional dietary components such as high [Ca] and/or phytate which reduce the bioavailability of dietary zinc may be necessary for significant cataract formation (Richardson et al. 1985).

High mortality is always associated with zinc deficiency (Ogino and Yang 1978; Wekell et al. 1983), although Richardson et al. (1985) were the only ones to categorically state that this occurred in the absence of any identifiable pathogen. We found that mortality was generally restricted to deficient fish and was invariably associated with pathology such as fin erosion, fungal infection, and nodular gill disease. In part, this might be due to reduced immune function, which is common in zinc-deficient mammals (see Fraker et al. 1986). On the other hand, nodular gill disease was, after 16 wk, most

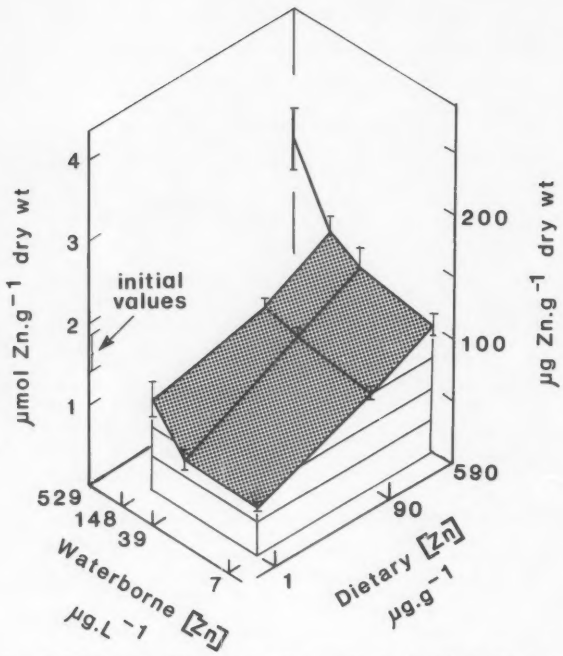

FIG. 3. Whole body [Zn] (µmol·g dry weight^{-1}) after 16 wk of treatment. Values are means ± SEM (9). Initital values (week 0) were 1.41 ± 0.05 µmol·g dry weight^{-1} (11).

prevalent in trout at ambient waterborne [Zn] regardless of dietary [Zn]. This suggests that elevated waterborne [Zn] itself may have had a prophylactic effect independent of the nutritional status of the fish, and warrants further study.

Concentration of all whole body elements (with the exception of copper) was elevated in deficient fish (Tables 2, 3). As noted earlier, this may have been a size effect, since these fish were significantly smaller than in other treatments, and negative correlations with size have been reported in wild fish (Wiener and Giesy 1979). Shearer (1984) however found positive correlations with size for most elements in laboratory-reared rainbow trout. Alternatively, a change in proximate body concentration may have accounted for the observed increases, especially for calcium, manganese, and vanadium where there was an elevation above initial values. Zinc-deficient fish had slightly higher ash weight and less lipid (Ogino and Yang 1978). Lipid has very low mineral concentrations, and high levels would tend to dilute elemental concentration (Shearer 1984).

A reciprocal relationship between whole body [Zn] and [Fe] has been noted by some authors (Ogino and Yang 1978; Wekell et al. 1986). In the present study this only occurred in deficient fish over the first dietary interval (1–90 µg·g^{-1}). Thereafter, the two responses paralleled each other. Increases in waterborne [Zn] resulted in a flat response for [Fe] across all dietary levels. Moreover, the pattern was the same for most other elements. These results do not support a causal relationship between zinc and iron over a broad range of dietary zinc.

Effects on Plasma and Whole Body [Zn]

There are relatively few measurements of plasma [Zn] in trout. Although mammalian values are about 0.01–0.02 mmol·L^{-1} (Underwood 1977), plasma [Zn] in fish is, for reasons which are unclear, frequently 10-fold higher (Bettger et al. 1987) and shows a higher range, 0.18–0.37 (Knox et al. 1984) and 0.28 (Zeitoun et al. 1977) falling to 0.06 ± 0.03 mmol·L^{-1} in rainbow trout fasted for 7 d (Spry and Wood 1984). Migratory (nonfeeding) sockeye salmon (*Oncorhynchus nerka*) had initial values of ~0.37 mmol·L^{-1}, which fell 50% over a month during spawning (Fletcher et al. 1975). In the present study, both the initial value (week 0, 0.15 mmol·L^{-1}) and all treatments at week 16 (with the exception of deficient fish) compared well with literature values for fish.

Plasma [Zn] after only 1 wk was a sensitive indicator of zinc restriction (Fig. 2A) and was depressed to quite low levels well before there was a treatment effect on growth or diseases appeared. By the end of the experiment, it was obvious that in the deficient fish, plasma [Zn] was extremely low (Fig. 2B) and growth had ceased. In fact, plasma [Zn] was a good indicator of growth, since it approximately mirrored the mean fish weight at the end of the experiment. These results indicated a broad plateau of zinc input (waterborne [Zn] = 7–148 µg·L^{-1}, dietary [Zn] = 9–590 µg·g^{-1}) over which plasma [Zn] remained unaltered, suggesting considerable homeostasis which was only overridden at extremes of very high or very low input.

Whole body [Zn] was less responsive than plasma [Zn] to changes in waterborne and dietary levels (compare Fig. 3 with 2B), although it was clearly affected by both. In the most deficient fish (waterborne [Zn] = 7 µg·L^{-1}, dietary [Zn] = 1 µg·g^{-1}) which had stopped growing, whole body [Zn] was ~0.6 µmol·g dry weight^{-1}. A similar *concentration*, but greater absolute load, was seen at the next higher waterborne [Zn] (39 µg·L^{-1}) where the fish grew nearly twice as large (Fig. 1 and 3). This concentration is likely the critical whole body concentration below which fish will not grow. Interestingly, Ogino and Yang (1978) found 0.43 µmol Zn·g dry weight^{-1} in zinc-deficient trout, and a similar range was found by Wekell et al. (1986) for trout on a zinc-deficient diet.

As dietary [Zn] increased, whole body [Zn] also increased, but in a log-linear fashion, with the result that fish retained much less zinc than they were being fed (see below). For example, at ambient waterborne [Zn], a nearly sevenfold increase in dietary [Zn] only increased whole body [Zn] by ~31%. The interesting fact here again is that when a waterborne zinc concentration was imposed in addition to diet, there were smaller, but nearly linear increases in whole body [Zn]. Some isolation thus exists between the two routes of uptake.

Calculations of dietary zinc retention (Fig. 4) indicated four major points. First, deficient fish had negative retention, indicating that these fish actually had lower zinc body burdens at week 16 than they did at week 0. This probably occurred because intestinal loss of zinc, which is large in mammals (Spencer et al. 1980; Weigand and Kirchgessner 1978), exceeded absorptive capacity. Absorptive capacity in flounder was only about 20% of various $ZnCl_2$ loads (Shears and Fletcher 1983). Second, trout on the deficient diet accumulated much larger loads than could be accounted for by diet alone, even assuming complete absorption, thereby yielding apparent retention figures well over 100% (i.e. 292, 665%). Nevertheless, the absolute increase in zinc load in the fish was less than that seen at any of the higher dietary [Zn]'s. A stimulatory effect of waterborne [Zn] on apparent zinc retention was also seen at the higher dietary levels, but to a much lesser extent (values in the 2–18% range). Third, as dietary [Zn] increased, trout retained much less on a percentage basis, until at dietary [Zn] = 590 µg·g^{-1}, apparent retention from the diet was only about 2%.

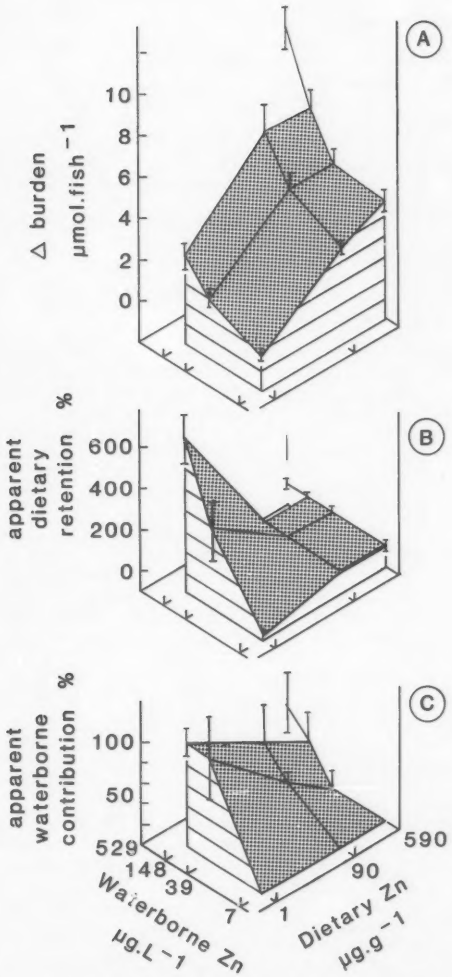

FIG. 4. (A) Change in body burden calculated as the difference in the means between the initital body burden (=0.99 μmol·fish^{-1} at week 0, n = 15) and that for ech treatment mean at 16 wk; (B) apparent dietary zinc retention; (C) apparent contribution by waterborne zinc to the total body burden. See text for details.

Fourth, at every dietary level, increases in waterborne [Zn] were associated with increased contributions from the waterborne source, with many values in the 31–57% range, even when dietary [Zn] was normal (90 μg·g^{-1}) or higher, and values of 100% when dietary [Zn] was deficient (1 μg·g^{-1}).

In view of this importance of zinc uptake from the water, by what route does it occur? One possibility is drinking of the medium, which might be especially important in fish on a low-zinc diet. Estimates of drinking rates for rainbow trout in freshwater range from nil (Shehadeh and Gordon 1969) to 1.43 μL·g^{-1}·h^{-1} (Lovegrove and Eddy 1982). Using the higher estimate, the highest waterborne [Zn] tested in fish on the deficient diet, 148 μg Zn·L^{-1}, would provide only 0.005 μg Zn·g^{-1}·d^{-1}. Food consumption of 3% body weight·d^{-1}, at dietary [Zn] = 1, 90, and 590 μg·g^{-1}, would give dietary inputs of 0.03, 2.7, and 17.7 μg Zn·d^{-1}. Even at the lowest dietary [Zn], the maximum input from imbibed water would only be 17% of the available dietary intake. Drinking therefore could only play a very small role in zinc uptake.

The other, far more likely, possibility is uptake across the gills. In acute exposures, the gill is always a site of high [Zn] (Joyner 1961; Hodson 1975; Bradley et al. 1985). Joyner's (1961) study further showed that esophageal wax plugs in brown bullheads (*Ictalurus nebulosus*) did not prevent accumulation of ^{65}Zn by several internal organs. Recently, we have shown that zinc is taken up from the water in a concentration-dependent fashion by intact adult trout, as well as by an isolated, perfused trout gill preparation (D.J. Spry and C. M. Wood, unpubl. data). Uptake by the intact trout was furthermore unaffected by esophageal ligation.

Our results clearly support homeostasis of internal zinc levels in the face of dietary and waterborne variation. For plasma, there was a broad region of regulation regardless of input, which was only depressed during extreme deprivation, and only elevated at very high dietary and waterborne input (Fig. 2A, 2B). Whole body [Zn] exhibited a rather less tightly controlled homeostasis in which there was no plateau region where concentration was independent of exposure. Perhaps "excess" whole body zinc was stored in a "sink" of lesser physiological importance (e.g. scales (Sauer and Watabe 1984) or bone). Nevertheless, over the same range where plasma showed a perfect plateau (Fig. 2B), whole body [Zn] exhibited less than two-fold variation in the face of 7- to 21-fold variation in the dietary and waterborne levels, respectively (Fig. 3). Indeed, on a whole body basis, apparent dietary retention was reduced to as low as 2% at high dietary [Zn] (Fig. 4). The true dietary retention would be even lower, since zinc was also entering from the water. This reduction may have been accomplished by decreased intestinal absorption, increased excretion, or both.

Studies on zinc accumulation in the field (cf. Bryan 1979; Giesy and Wiener 1977; Wiener and Giesy 1979; Roch et al. 1982; Saltes and Bailey 1984) support the concept of zinc homeostasis in fish, as has been well-documented in mammals (e.g. Cousins 1985). In laboratory exposures, whole body and tissue levels have risen, but only slightly (two- to threefold), when waterborne [Zn] has increased several hundred fold (Farmer et al. 1979; Holcombe et al. 1979). Steady-state concentrations which have been observed may in part be due to growth dilution (Spehar 1976; Farmer et al. 1979), although active excretion has been proposed (Pierson 1981). It is still not clear how closely whole body zinc is regulated, or whether this is effected by active or passive mechanisms which maximize net zinc uptake in deficiency and limit net uptake when excess zinc is present in the water or diet.

In summary, a zinc-deficient diet induced a zinc deficiency in trout when supplementary zinc was not available from the water. Elevations of waterborne [Zn] to 39 and 148 μg·L^{-1} partially corrected the deficiency but did not restore either plasma or whole body [Zn] to levels seen either initially or in fish raised for 16 wk on a zinc-adequate diet. At elevated waterborne [Zn], zinc was taken up from the water regardless of the dietary load. Waterborne contribution was up to 57% of the increased burden on zinc-adequate diets, despite the fact that dietary zinc was three orders of magnitude higher. At waterborne [Zn] most commonly encountered in the wild (<10 μg Zn·L^{-1}), waterborne contributions to whole body [Zn] are likely to be insignificant. Nonlethal waterborne [Zn] which may be encountered as a result of pollution (e.g. 39–529 μg·L^{-1}) may cause considerable uptake from the water. Elevation of waterborne [Zn] to even 500 μg·L^{-1} was not stressful to fingerling

trout based upon growth and mortality, and may have been prophylactic against waterborne pathogens. Plasma [Zn] was relatively constant over most of the range of zinc input, whereas whole body [Zn] reflected input from both sources, but in an attenuated fashion. Metabolism of other elements did not appear to be directly affected by zinc treatments, and there were no toxic effects.

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Response of Recently Metamorphosed Anadromous Parasitic Lamprey (*Lampetra tridentata*) to Confinement in Fresh Water

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Clarke, W. C., and R. J. Beamish. 1988. Response of recently metamorphosed anadromous parasitic lamprey (*Lampetra tridentata*) to confinement in fresh water. Can. J. Fish Aquat. Sci. 45: 42–47.

Recently metamorphosed *Lampetra tridentata* were collected from six rivers in British Columbia in early autumn and held in fresh water in the laboratory at ambient temperature and simulated natural photoperiod. Ability to survive in fresh water varied considerably among populations, ranging from the Babine River population which did not survive beyond February to the Chemainus River population which survived until July. Associated with the onset of mortality was a decrease in plasma sodium concentrations. Plasma sodium concentrations in individual lampreys were correlated with condition factor, indicating that osmoregulatory failure may have resulted from depletion of body energy reserves. In a separate experiment, postmetamorphic lamprey sampled from two rivers fed poorly in fresh water and feeding did not prolong their survival. We conclude that confinement of *L. tridentata* in fresh water does not easily result in the formation of landlocked populations.

Des *Lampetra tridentata* récemment métamorphosées ont été capturées dans six rivières de la Colombie-Britannique au début de l'automne et gardées en laboratoire dans les conditions suivantes : eau douce, température ambiante et photopériode simulant la photopériode naturelle. La capacité de survie en eau douce a varié considérablement selon les populations, les extrêmes étant la population de la rivière Babine qui n'a pas survécu après février et la population de la rivière Chemainus qui a survécu jusqu'en juillet. Le début de la mortalité coïncidait avec une baisse du taux de sodium plasmatique. Les taux de sodium plasmatique chez les lamproics individuelles étaient corrélés avec le coefficient de conditions, ce qui indique que l'insuffisance de l'osmorégulation pourrait résulter de l'épuisement des réserves énergétiques de l'organisme. Dans une autre expérience, des lamproics métamorphosées provenant de deux rivières s'alimentaient peu en eau douce et l'alimentation n'a pas prolongé leur survie. On conclut donc que le confinement de *L. tridentata* en eau douce n'aboutit pas facilement à la formation de populations landlockées.

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Anadromous parasitic lamprey enter salt water after metamorphosis where they feed on other fishes. Entry into salt water can occur almost immediately after metamorphosis, be delayed for up to 10 mo from the onset of metamorphosis, or occur in two stages (Beamish and Potter 1975; Potter et al. 1980; R. J. Beamish 1980; Maitland et al. 1984). Migration into salt water is associated with increased stream discharge (Potter 1970; Potter and Huggins 1973; Beamish and Youson 1987) and with the opening of the foregut during metamorphosis (Hardisty et al. 1970; Potter et al. 1980; Richards and Beamish 1981; Beamish and Youson 1987).

Although there have been studies of the ability of anadromous lamprey to adapt to salt water (Potter and Huggins 1973; F. W. H. Beamish 1980; Beamish and Youson 1987), there are very few reports of the consequences of retaining an anadromous species in fresh water after metamorphosis (Beamish and Youson 1987; R. J. Beamish 1980). Such studies are relevant because it is generally believed that freshwater parasitic lamprey arise when anadromous species invade a new freshwater habitat or are prevented from entering the sea. Because of the abundance of anadromous parasitic lampreys and the adverse effect that freshwater parasitic lamprey can have on other fishes (Applegate 1950; Birman 1950; Nikol'ski 1956; Gritsenko 1968; Beamish 1982; Smith and Tibbles 1980; Holcik 1986), it is important to know if the confinement of postmetamorphic anadromous parasitic lamprey in fresh water could result in the establishment of freshwater feeding for the duration of the trophic phase. This knowledge is particularly important in areas such as British Columbia where *Lampetra tridentata* is common (R. J. Beamish, unpubl.) and there are numerous proposals for the construction of hydroelectric dams.

There are two species of anadromous parasitic lamprey in British Columbia (*L. ayresi* and *L. tridentata*). Recently, Beamish and Youson (1987) showed that it was possible to keep a few *L. ayresi* in fresh water for their entire postmetamorphic phase. This study examines the response of *L. tridentata* from a number of populations to confinement in fresh water.

Materials and Methods

In September 1981, samples of recently metamorphosed *L. tridentata* were collected from the Babine River. Twenty-five

lamprey were acclimated to salt water at ambient temperature over a 3-d period in November. Juvenile coho (*Oncorhynchus kisutch*) and sockeye salmon (*O. nerka*) were added to the tank to provide food. The number of lamprey that were attached to salmon were noted daily for the first month and dead salmon were replaced. Another 48 lamprey were held in a separate tank of fresh water without food and samples of five lamprey were taken each month for analysis of plasma sodium concentrations. Blood was collected into heparinized microhematocrit tubes from a transverse incision behind the last branchial opening (Beamish et al. 1978). The tubes were centrifuged to separate the plasma which was diluted for analysis of sodium concentrations (Blackburn and Clarke 1987). Lamprey were measured at the beginning of the experiment and at the end of February 1982.

In September 1982, samples of recently metamorphosed lamprey were again collected in the Babine River. Half were held in fresh water without food and samples of five lamprey were taken monthly for analysis of plasma sodium concentrations. Another 20 lamprey were held in a separate tank of fresh water; small Pacific herring (*Clupea harengus pallasi*) were added to provide food. Herring were used as prey in the freshwater experiment to avoid the problem of fungus infections which may be induced by nipping of the lamprey by juvenile salmon. The number of lamprey that were attached to the herring were noted daily and dead herring were replaced. A second sample of 25 recently metamorphosed lamprey from the Big Qualicum River was held in fresh water with herring provided as food.

Recently metamorphosed lamprey were collected in September and early October 1983 from six rivers in British Columbia (Fig. 1) using electroshockers and transported to the laboratory. Lamprey from each population were distributed among three tanks in mid-October. The number of lamprey at the start of the study ranged from 145 for the Babine River population to 66 for the Kanaka Creek population. Sand and rocks were placed on the bottom to provide suitable shelter to reduce the amount of movement. Since provision of prey failed to enhance survival in the 1982 experiments, lamprey were held without food under simulated natural photoperiod at ambient water temperature (see Table 2). Samples of five lamprey from each population were taken at approximately monthly intervals from mid-October until February and at approximately 3-wk intervals thereafter; they were anesthetized in 2-phenoxyethanol, blotted dry on a damp chamois, and measured in length and weight. Condition factor was calculated as weight (g)/length $(mm)^{2.08} \times 10^6$. The exponent 2.08 calculated from a regression of log weight on log length was used instead of the power 3 used by Potter and Beamish (1977). Six 24-h seawater challenge tests (Blackburn and Clarke 1987) were performed at approximately monthly intervals from November to June using lamprey from the Big Qualicum, Puntledge, and Babine populations to determine their ability to adapt to seawater. Samples of five lamprey were transferred directly from the freshwater holding tanks to a tank of flowing seawater at the same temperature.

Results

In the 1981 experiment, recently metamorphosed Babine River lamprey acclimated to 30‰ seawater commenced feeding on November 25, 9 d after juvenile salmon were provided. Thereafter, feeding occurred daily. Apart from a single mortality in November, no further deaths occurred in salt water for the duration of the experiment through February 1982. During this period, the lamprey grew an average of 0.6 cm.

In the 1982 experiment, Babine River lamprey held in fresh water commenced feeding on October 28, 1 d after juvenile herring were presented. Some feeding occurred almost daily during November and early December. Feeding was less frequent thereafter. The number of attacks and the average daily herring mortalities per lamprey were considerably lower than in lamprey feeding in salt water. The first lamprey died on November 8, half were dead by mid-December, and all were dead by December 29. All moribund lamprey had fungus which could not be cured by malachite green treatments. Big Qualicum River lamprey held in fresh water commenced feeding on December 3, 1 d after being presented with live herring. Feeding continued daily until late December and then declined. The first lamprey died on December 9, half were dead by mid-January 1983, and all were dead by January 21, 1983.

The mean length of lampreys for all six populations in the 1983 experiment was 13.2 cm; at 15.2 cm, the Babine River population was largest and differed significantly ($p < 0.05$) from the smallest group from the Puntledge River (12.3 cm). Mean weight ranged from 3.4 g for the Babine River population to 2.4 g for the Somass River one. The average condition factor for all populations was 1.44 in mid-October, declining to 1.30 in the November sample; it differed significantly among populations, ranging from 1.41 in the Puntledge River to 1.17 in the Babine River population (Table 1).

There were no mortalities during the first month of the 1983 study, with the exception of a single lamprey from the Puntledge River population. Mortalities increased sharply in the Babine River population during the second half of November and continued until mid-February when all fish had died (Fig. 2). Mortalities in the Somass River and Kanaka Creek populations commenced early in December and most fish died by mid-March. Mortalities for the other populations started later and occurred over a longer period. Mortality was most delayed in the Chemainus River population where it increased in late April and peaked in July. In most populations, mortality rose to a maximum and then declined until the remaining animals died. Cumulative percent mortality to the end of March was highest in populations with the lowest average plasma sodium concentration from October to March (Spearman Rank correlation coefficient 0.93, $n = 6$, $p < 0.05$). The Babine and Somass River populations also had the lowest condition factor; however, the overall Spearman rank correlation coefficient between mean condition factor from October to March and cumulative percent mortality to the end of March ($r_s = 0.47$, $n = 6$) was not significant.

Plasma sodium concentrations did not differ among populations in mid-October 1983; the average for all six populations was 120 mmol/L (Table 2). Plasma sodium levels declined significantly in January in four populations but not in the Puntledge and Chemainus River samples (Table 2). Lampreys in the Babine River population were the least able to maintain blood sodium concentrations in fresh water, followed by those in the Somass River, Kanaka Creek, and Big Qualicum River populations. Samples of the Babine River population taken in 1981, 1982, and 1983 exhibited a consistent pattern of declining plasma sodium levels during November and December followed by low and variable levels during December to February (Fig. 3). No significant decline in plasma sodium concentrations was observed in the Puntledge River population; the mean for 12 samples taken from October to July was 109.5 mmol/L

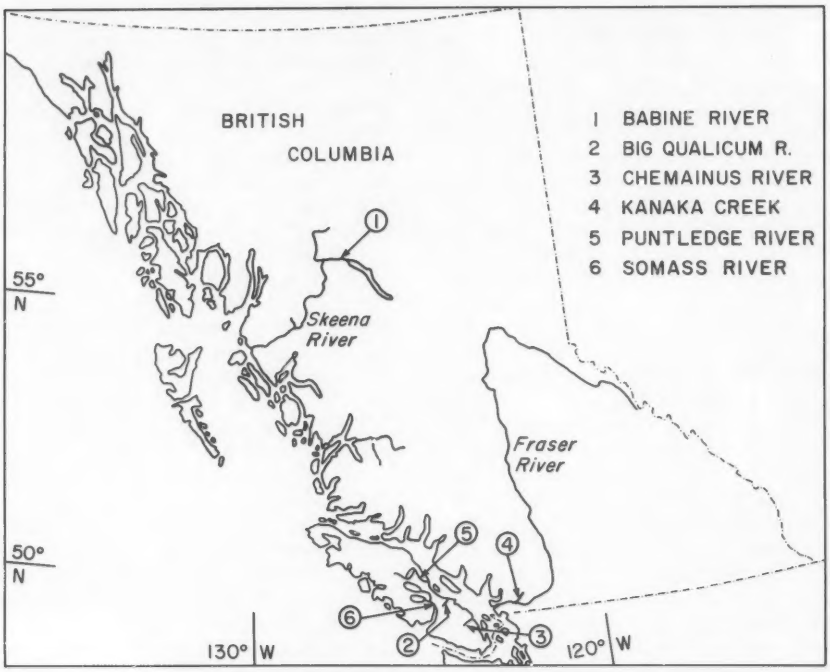

FIG. 1. Locations of British Columbia rivers from which lamprey were collected.

TABLE 1. Condition factor of lamprey from six populations held in the laboratory and sampled from October to March. Values are arithmetic mean (SE) of five fish per group (except March Babine sample where $n = 1$). Population or monthly means followed by the same letter are not significantly different by Tukey's Studentized range test at the 0.05 level.

	October	November	January	February	March	Means
Big Qualicum R.	1.68 (0.10)	1.36 (0.10)	1.36 (0.11)	1.38 (0.07)	1.32 (0.09)	1.42 a
Puntledge R.	1.59 (0.05)	1.49 (0.05)	1.39 (0.06)	1.30 (0.02)	1.30 (0.07)	1.41 a
Kanaka C.	1.46 (0.09)	1.36 (0.06)	1.49 (0.07)	1.31 (0.06)	1.27 (0.02)	1.38 ab
Chemainus R.	1.35 (0.08)	1.27 (0.03)	1.31 (0.04)	1.26 (0.04)	1.19 (0.04)	1.27 bc
Somass R.	1.38 (0.05)	1.20 (0.03)	1.10 (0.10)	1.14 (0.06)	1.09 (0.08)	1.18 c
Babine R.	1.19 (0.08)	1.11 (0.07)	1.26 (0.06)	1.15 (0.05)	0.98	1.17 c
Mean	1.44 a	1.30 b	1.32 b	1.26 b	1.22 b	

with a minimum of 102 mmol/L in January. Over the same period, the mean plasma sodium concentration measured in the Chemainus River population was 113 mmol/L; a minimum of 101 mmol/L was reached in late June but this was not a statistically significant drop. For all six populations, sodium concentrations were positively correlated with monthly mean holding temperature ($r_s = 0.90$, $n = 5$, $p = 0.05$) and condition factor ($r = 0.25$, $n = 135$, $p < 0.01$) but negatively correlated with length ($r = -0.272$, $n = 135$, $p < 0.01$). However, there was no significant correlation between sodium concentrations and body weight ($r = -0.099$, $n = 135$). The mean plasma magnesium concentration in freshwater lamprey was 0.85 mmol/L; it did not differ significantly among populations and was not significantly correlated with plasma sodium ($r = 0.075$).

Seawater adaptability did not differ appreciably among the three populations tested (Table 3). However, all three exhibited a decline in seawater adaptability at the time of the January challenge test as evidenced by elevated plasma sodium concentrations. The Big Qualicum River population suffered mortality after seawater challenge in January, February, and March. In contrast, the Puntledge River population showed no mortality until the June challenge test.

Discussion

This is the first study comparing the ability of metamorphosed lamprey from different populations of an anadromous species to survive in fresh water. The only known report of anadromous parasitic lamprey being kept to maturity in fresh water is for *L. ayresi* from one population in the Fraser River (Beamish and Youson 1987). Other laboratory studies have shown that it is difficult to maintain metamorphosed *Petromyzon marinus* (Potter and Beamish 1977) and *Geotria australis* (Potter et al. 1980) in fresh water. Our 1983 study showed that the ability of metamorphosed *L. tridentata* to survive in fresh water differed considerably among the populations tested. The most dramatic difference occurred between the Babine River population and the Chemainus River population. No

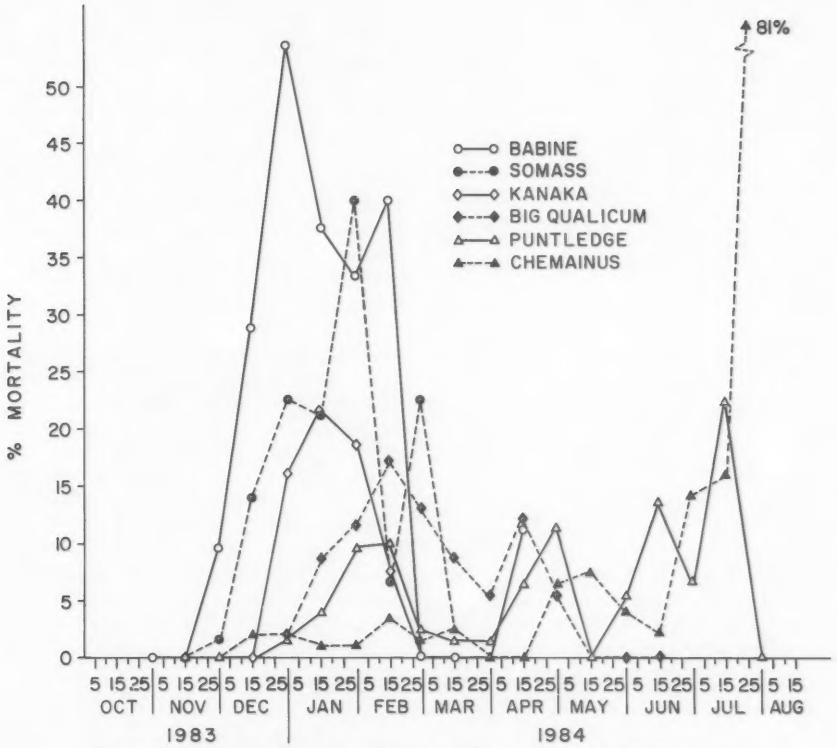

FIG. 2. Biweekly mortality in six populations of *L. tridentata* held in fresh water.

TABLE 2. Plasma sodium concentrations (mmol/L) in lamprey from six populations held in the laboratory and sampled from October 1983 to March 1984. Values are arithmetic mean (SE) of five fish per group (except March Babine River sample where $n = 1$). Population or monthly means followed by the same letter are not significantly different by Tukey's Studentized range test at the 0.05 level.

	October	November	January	February	March	Means
Babine R.	119.5 (4.2)	102.8 (4.6)	75.7 (5.6)	86.0 (12.2)	93.4	95.4 a
Somas R.	124.7 (2.4)	116.1 (3.7)	93.2 (5.0)	101.4 (7.5)	95.5 (8.8)	106.2 b
Kanaka C.	120.1 (2.0)	118.3 (2.4)	79.1 (10.0)	112.6 (10.6)	103.9 (5.7)	106.8 b
Big Qualicum R.	123.9 (3.6)	120.5 (1.1)	103.3 (5.9)	97.7 (4.1)	103.0 (3.7)	109.7 bc
Puntledge R.	116.3 (1.2)	123.7 (1.7)	101.9 (6.7)	111.7 (7.2)	110.8 (2.2)	112.9 bc
Chemainus R.	117.1 (3.0)	119.5 (2.5)	110.1 (8.5)	122.0 (5.5)	121.7 (6.6)	118.1 c
Mean	120.2 a	116.8 a	93.9 b	105.2 c	104.7 c	
Mean holding temperature (°C)	10.6	9.1	4.5	5.6	6.9	

Babine River lamprey survived beyond mid-February, while most of the Chemainus River lamprey survived until July. Possibly this variation in ability to survive in fresh water results from adaptations for different seasonal timing of downstream migration among local populations.

Because food was not provided and temperature was not controlled, it was not determined if any of the lamprey in the 1983 experiment, particularly those of the Chemainus River population, could survive in fresh water if given the opportunity to feed. However, the 1982 experiment demonstrated that feeding by lamprey from the Babine and Big Qualicum rivers in fresh water was not as intense as that observed for lamprey of the same age transferred to seawater in 1981; despite some feeding, these groups did not survive as long in fresh water as unfed ones from the same populations in the 1983 experiment. Our results are in agreement with those of Richards and Beamish (1981) who reported that lamprey from the Chemainus River failed to feed in fresh water during a 75-d experiment, while other groups transferred to salt water fed well.

Mortality was associated with a decline in plasma sodium concentrations. The ability to maintain plasma sodium concentrations in fresh water was independent of body weight but was negatively correlated with length. One possible cause of the observed decline in plasma sodium concentrations is depletion

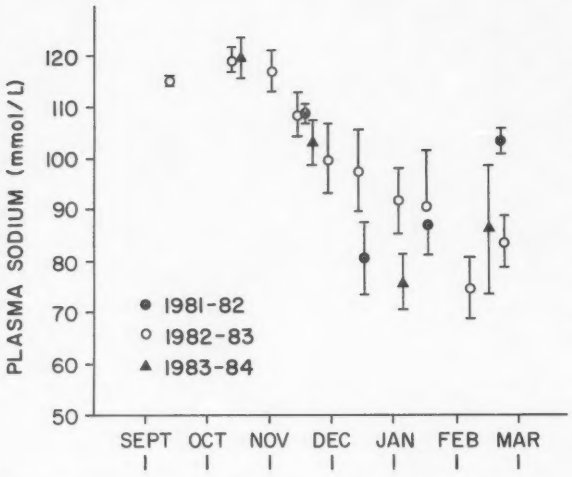

FIG. 3. Plasma sodium concentrations in Babine River lamprey held in fresh water in the laboratory in 1981–82, 1982–83, and 1983–84.

of body lipid reserves. Depending on timing of entry into salt water, anadromous parasitic lamprey may spend up to 10 mo in fresh water without feeding before they enter salt water (Beamish et al. 1979; Gritsenko 1968; Potter et al. 1980; Potter and Beamish 1977; Beamish and Youson 1987). A histological study of *P marinus* revealed that lipid levels declined through the winter and were extremely low in young adults at the end of the nontrophic period in May (Youson et al. 1979). Beamish et al. (1979) measured a lipid level of only 1.3% in a similar group of *P. marinus*. In the present study, we did not measure body lipid levels, but all populations exhibited a significant decline in condition factor during the experiment which suggests that lipid levels also decreased. Condition factor was correlated with plasma sodium concentration and all populations maintained similar sodium concentrations in October; the difference in timing of the decline in sodium concentrations and subsequent increase in mortality may be related to the time at which body lipid reserves became depleted in the various populations. The sharp drop in plasma sodium concentrations in early January may have resulted from the low water temperature at this time. A possible explanation for variation in plasma sodium levels among stocks after October is that there are genetic differences in osmoregulatory ability among populations. Differences in osmoregulatory ability are known between landlocked and anadromous *P. marinus* (Mathers and Beamish 1974) and between *L. tridentata* and the closely related *L. macrostoma* (R. J. Beamish 1980).

Mortalities in the anadromous *L. ayresi* held in fresh water increased sharply in June, about 2 mo after the opening of the foregut and about 10 mo after the onset of metamorphosis (Beamish and Youson 1987). This species normally enters the ocean in May and June and commences feeding immediately (R. J. Beamish 1980; Beamish and Youson 1987). The sharp increase in mortality in fresh water in mid-June coincided with the initiation of feeding in salt water and may be related to the depletion of lipid reserves as we have suggested for *L. tridentata* in the present study.

Variation in the timing of opening of the foregut could contribute to osmoregulatory variability (F. W. H. Beamish 1980). However, in the Chemainus River population which survived the longest, the foregut is known to be open by September (Richards and Beamish 1981). Furthermore, R. J. Beamish (1980) has shown that *L. tridentata* from five populations including those from the Babine and Big Qualicum rivers feed by the end of October when held in salt water in the laboratory. In the present study, lamprey from Big Qualicum, Puntledge, and Babine rivers were able to hypoosmoregulate well in a

TABLE 3. Plasma sodium concentrations (mmol/L) and mortality of lamprey 24 h after transfer to 29‰ seawater. Values are arithmetic mean (SE) of surviving fish in sample of five for each test.

	Population		
	Big Qualicum R.	Puntledge R.	Babine R.
November 22			
Plasma sodium	123.7(2.9)	122.1(1.4)	124.1(2.0)
Mortality	0/5	0/5	0/5
January 5			
Plasma sodium	153.3(8.4)	148.5(13.7)	138.3(9.1)
Mortality	1/5	0/5	0/5
February 16			
Plasma sodium	129.4(17.1)	117.0(2.9)	—
Mortality	1/5	0/5	—
March 20			
Plasma sodium	134.8(9.5)	117.8(0.8)	—
Mortality	2/5	0/5	—
April 26			
Plasma sodium	145.6(13.4)	139.5(17.6)	—
Mortality	0/5	0/5	—
June 8			
Plasma sodium	—	143.8(15.9)	—
Mortality	—	1/5	—

24-h seawater challenge test at the end of November, indicating that the foregut was open (Richards and Beamish 1981). Accordingly, we believe that it is unlikely that variability in the time of the opening of the foregut contributed significantly to the ability of the various populations to survive in fresh water.

From our results, it is evident that postmetamorphic *L. tridentata* sampled from six populations in British Columbia do not readily remain in fresh water. However, we cannot exclude the possibility that a very few individuals from a population could survive and feed in fresh water; it seems possible that the closely related freshwater parasitic species *L. macrostoma* (Beamish 1982) and *L. minima* (Bond and Kan 1973) evolved in this manner. Our conclusion that the confinement of *L. tridentata* in fresh water does not easily result in the formation of landlocked populations is corroborated by the observation that no landlocked populations of *L. tridentata* have been found in British Columbia (R. J. Beamish, unpubl. obs.) and only a few have been reported throughout its entire range (Hubbs 1971; Vladykov and Kott 1979).

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Independent Distributions of Fish Larvae and Their Prey: Natural Paradox or Sampling Artifact?

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Several recent studies have concluded that larval fish distributions are independent of the abundance and distribution of their prey. All of these studies used coarse-mesh (>250 μm) nets, incapable of retaining the edible zooplankton for fish larvae, to provide quantitative estimates of the larval food resource. The assumption that zooplankton captured by coarse-mesh nets provided a reliable index of the edible zooplankton for fish larvae was tested and unsupported by the analysis of several independent data sets. In the waters off southwestern Nova Scotia the biomass of edible zooplankton for young larval fish was highly concentrated in the nearshore region, progressively lower levels were evident offshore on the shelf, and the mesoscale distributional pattern did not accurately reflect the total zooplankton biomass retained by a 333-μm-mesh net. Independent spatial distributions of discrete size groups of zooplankton are characteristics similar to zooplankton distributions reported for other geographic regions. A more logical explanation for the reports of "paradoxical" distributions of fish larvae and their prey is to be found in the inefficiency and bias in the sampling methods used to evaluate the larval food resource. Failure to properly evaluate the larval food resource has led to inappropriate testing of some long-standing hypotheses in fisheries biology.

Plusieurs études récentes ont permis de conclure que la distribution des poissons larvaires est indépendante de l'abondance et de la distribution de leurs proies. Dans toutes ces études, on a utilisé des filets à grosses mailles (>250 μm) incapables de retenir le zooplancton consommable par les poissons larvaires pour estimer quantitativement les ressources alimentaires des poissons larvaires. L'hypothèse selon laquelle le zooplancton capturé dans les filets à grosses mailles constitue un indicateur fiable du zooplancton qui est consommable par les poissons larvaires a été testée et rejetée par l'analyse de plusieurs séries de données indépendantes. Au sud-ouest de la Nouvelle-Écosse, la biomasse de zooplancton consommable par le jeune poisson larvaire était très concentrée près de la côte, mais diminuait graduellement en direction du large (sur le plateau continental); la distribution à l'échelle moyenne ne reflétait pas avec précision la biomasse totale de zooplancton retenue dans le filet à mailles de 333 μm. Les distributions spatiales indépendantes de groupes de taille discrets sont similaires aux distributions de zooplancton rapportées pour d'autres régions géographiques. Une explication plus logique pour ces distributions « paradoxales » de poissons larvaires et leurs proies pourrait résider dans l'inefficacité et l'erreur des méthodes d'échantillonnage utilisées pour l'évaluation des ressources alimentaires des poissons larvaires. Un certain nombre d'hypothèses formulées depuis longtemps dans le domaine de la biologie des pêches n'ont pu être vérifiées de façon appropriée du fait que l'on a mal évalué les ressources alimentaires des poissons larvaires.

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Both natural and artificial factors can influence quantitative estimates of the biomass and distribution of zooplankton. Natural factors can influence zooplankton biomass directly through production processes or indirectly through structural changes in zooplankton size or species composition. Natural factors that act directly on zooplankton biomass include local feeding conditions (Mullin and Brooks 1976; Durbin et al. 1983), predation (Koslow 1981; Frank 1986), water temperature (Reid et al. 1958), and currents (Wickett 1967; Chelton et al. 1982). Structural or qualitative changes in zooplankton assemblages that result in variation in biomass have commonly been linked to size-selective predation (Hall et al. 1976) and water temperature (Deevey 1960; Ware 1977). Interactive effects among these natural factors also occur (Warren et al. 1986).

Artificial factors known to influence quantitative estimates of zooplankton biomass include sampling design, collecting, and processing methods, and data presentation (Brinton 1962; Green 1979). Biases caused by these artificial factors are most serious when the target population is undefined which frequently leads to an inappropriate choice of equipment and methods for sampling (Omori and Hamner 1982). This reality is often ignored and biased samples are used to make quantitative assessments of zooplankton (Omori and Ikeda 1984). The result is an unrealistic picture of temporal and spatial zooplankton distributions and abundance. It is essential that sampling effects be distinguished from natural ones before ecological relationships are explored.

Ichthyoplankton studies appear to be particularly prone to bias in the quantitative assessment of the planktonic food

resource available to fish larvae. The most common problem is the use of plankton sampling gear having net mesh sizes that exceed the prey size requirements of the larvae. Generally, fish larvae <10 mm consume prey particles <200 μm (Arthur 1977; Theilacker and Dorsey 1980) whereas the net mesh sizes commonly used to collect both ichthyoplankton and zooplankton exceed 200 μm. The utility of zooplankton data obtained by these means to studies of larval fish/food relationships hinges on the assumption that the edible biomass available to the larvae is correlated with the larger zooplankton retained by the coarse-mesh net.

Several examples of larval fish species exhibiting spatial and temporal distributions independent of their planktonic prey have recently been reported in the literature (cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), pollock (*Pollachius virens*), silver hake (*Merluccius bilinearis*), O'Boyle et al. 1984; herring (*Clupea harengus*), Sinclair and Iles 1985; Pacific mackerel (*Scomber japonicus*), Sinclair et al. 1985; sandlance (*Ammodytes* sp.), sea snail (*Liparis* sp.), redfish (*Sebastes* sp.), Anderson and Gardner 1986). The general conclusion from these studies was that larval fish abundances were independent of the abundance and distribution of their prey. Sinclair and Iles (1985) have extended this conclusion to propose a new theory that stresses the importance of physical oceanographic processes over biological interactions to account for spatial patterns of larval fish distributions. Sinclair and Iles (1985) hypothesized that food conditions in the larval distributional areas are of secondary importance to survival and that larval retention in a geographically predictable area is the most important factor regulating the success of the reproductive unit. The common characteristic of all of the above-cited studies was their use of coarse-mesh nets to evaluate the distributions of both the larval fish and their zooplankton prey. Despite this potentially serious bias, some of these studies have challenged two long-standing hypotheses that link food production dynamics to larval fish survival and recruitment (i.e. the critical period and match/mismatch hypothesis: Hjort 1914 and Cushing 1972, respectively).

In this paper I assess the extent to which biases produced by collection methods have influenced the outcome of studies reporting independent distributions of fish larvae and their prey. I draw on published and newly gathered zooplankton data collected with fine- and coarse-mesh nets to examine the validity of the assumption that the zooplankton retained by coarse-mesh nets provides a reliable index of food available to fish larvae.

Materials and Methods

During May of 1985 and 1986 I examined the spatial distribution of zooplankton biomass from the coastal and continental shelf waters off southwestern Nova Scotia. The study area overlapped geographically with one of the previously published studies in question (southwestern Scotian Shelf, O'Boyle et al. 1984) and was directly adjacent to the other (Gulf of Maine – Bay of Fundy, Sinclair and Iles 1985). This permitted a direct evaluation of bias associated with collection methods typically used to quantify the distribution of larval fish and their food. The month of May was chosen for intensive study because it follows the period of peak spawning by haddock and cod (Scott 1983; O'Boyle et al. 1984). These species were claimed by O'Boyle et al. (1984) to show spatial and temporal distributions independent of those of their food supply.

Sampling stations were distributed along four cross-shelf transects during 1985 and 1986 (Fig. 1). Total depth at the stations ranged between 40 and 192 m (mean = 88 m) and station distance from land (Wedgeport, N.S.) varied between 32 and 141 km. The grid was sampled during 8–23 May and 9–20 May in 1985 and 1986, respectively.

Two gear types were deployed at each station (Table 1). A small-scale version of the BIONESS (for details see Frank 1986) was used to sample zooplankton at up to seven discrete depths per station. The number of depths sampled (range = 4–7) was dependent on the total depth at the station. On average, 70% of the water column was sampled at each station. In both years, sample depths (± 1–3 m depending on sea state) were 5, 10, 20, and 30 m (1986 only) and 35, 45, 55, and 65 m (1985 only). Each depth stratum was sampled for a 7- to 8-min period. The mouth area of each of the seven 333-μm-mesh nets was 0.25 m^2. Towing speeds generally averaged 1.5 m/s. The volume of water filtered by each net was not corrected for filtration efficiency which averaged 77% (SD = 4.98) and 84% (SD = 4.61) for 1985 and 1986, respectively. At the termination of the BIONESS tow the ship held position for multiple vertical hauls of a 0.75-m-diameter, 80-μm-mesh ring net (Table 1). The 80-μm net retains small zooplankton types known to be prey of cod and haddock larvae (Kane 1984) which dominate the ichthyoplankton of the region sampled during the late winter – early summer period. The bridle of the 80-μm net, a nylon rope secured to the stainless steel frame, and a nylon rope noose located 20 cm below the mouth of the net were attached to a General Oceanics double trip mechanism. I sampled discrete depths from the surface to 20 m, 20–40 m, and, where total depth permitted, 40–60 m (1985 only). A metered block was used to determine depth. Towing speeds were approximately 0.5 m/s.

On deck, all plankton samples, from both gear types, were treated identically. The contents of each cod end were first passed through a 4-mm-mesh sieve to remove all large forms of macrozooplankton (e.g. jellies, chaetognaths, shrimps, etc.). The macrozooplankton and the remainder of the sample were placed into separate containers and preserved in 5% formalin buffered with sodium borate.

In the laboratory, that portion of the sample that had passed through the 4-mm-mesh sieve was sorted under a binocular dissection microscope to remove all ichthyoplankton and the smaller macrozooplankton (e.g. chaetognaths, tomopterans, decapods, euphausiids, amphipods). The balance of the sample, composed principally of copepods, was fractionated into several size categories (Table 1) to provide a quantitative measure of the size composition of the plankton (Ware 1977; Hargrave et al. 1985; Frank 1986). BIONESS samples were fractionated into four size categories by washing through a graded series of screens (1050, 860, 602, 308 μm) stacked from largest to smallest. Three additional size categories (80–153, 153–308, >308 μm) were processed from the vertical tow samples (Table 1). Precautions were taken to minimize the bias associated with size sorting of zooplankton on standing screens. The majority of the samples were quantitatively subsampled (one eighth to one half of the total sample volume) using a Motoda box type plankton splitter to reduce the amount of material processed at any one time. This prevented clogging of the screens and no water accumulation was noticed on any of the screens during rinsing. Thorough rinsing involved application of a steady stream of tap water directed at the top of each screen to ensure adequate sorting of the material. All size fractions were then

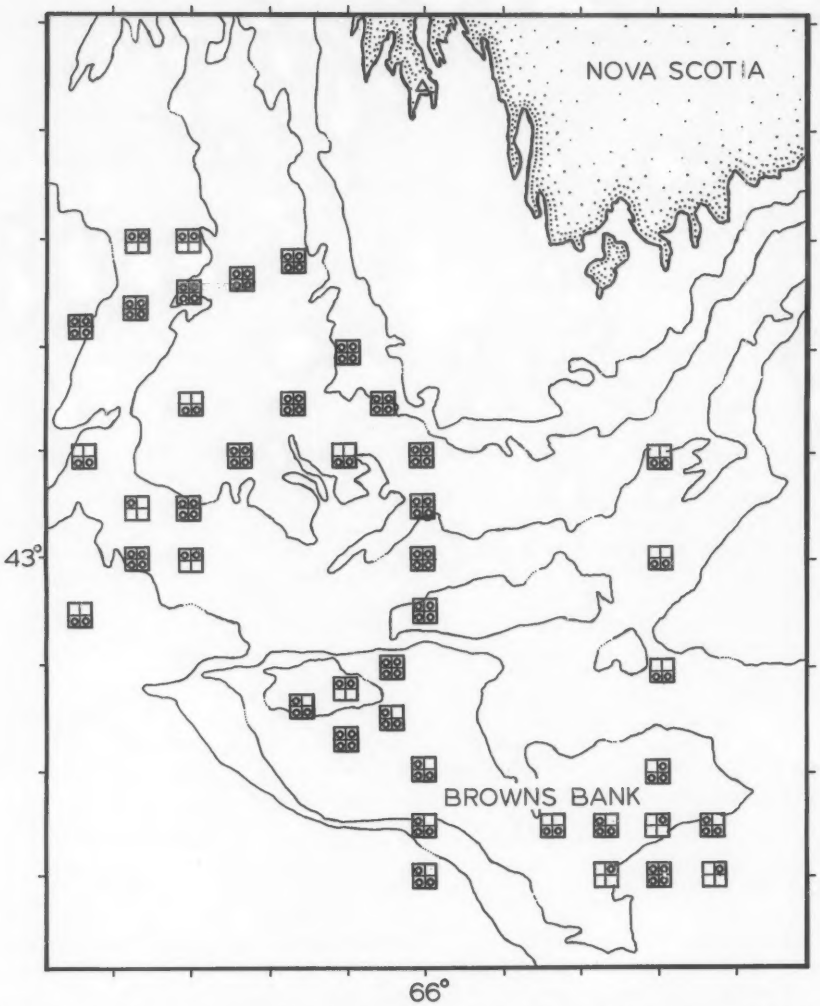

FIG. 1. Coastal and shelf region off southwestern Nova Scotia. Station positions (designated by center of matrix) are shown for the 1985 and 1986 May cruises. Open circles in cells of matrix indicate gear type used at each station and year of deployment (left column = BIONESS, right column = vertical net; top row = 1985, bottom row = 1986). Depth contours are in fathoms (1 fathom = 1.83 m). Δ = location of Wedgeport, N.S.

rinsed with freshwater, resuspended, and filtered by aspiration through a preweighed glass microfiber filter. The filters were oven dried at 70°C for 24 h, removed to a desiccator, and weighed to an accuracy of 10 μg. The dry weight biomass of each size fraction was determined by difference. All dry weight determinations were expressed as milligrams per cubic metre.

The mass of the size-specific zooplankton fractions at each station was expressed as the mean of the multiple depth strata. Correlation analysis was used to (1) determine the similiarity of the biomass of equivalent size fractions obtained with the two types of gear, (2) test the assumption that the biomass of larger zooplankton retained by the coarse-mesh net (333 μm in my study) provided a reliable index of the biomass of smaller edible zooplankton, and (3) determine whether consistent horizontal patterns existed in the biomass distribution of large and

TABLE 1. Summary of gear types and net mesh sizes used to sample zooplankton at multiple depth strata at selected stations (*n*) in the sea off southwestern Nova Scotia. Filtered volumes per stratum are approximate values. Key to zooplankton size fractions that were processed in the laboratory: 1 = >1050 μm; 2 = 860–1050 μm; 3 = 602–860 μm; 4 = 308–602 μm; 5 = 153–308 μm; 6 = 80–153 μm; 7 = >308 μm.

Year	Gear type	Mesh size (μm)	Volume (m^3)	*n*	Size fractions
1985	BIONESS	333	150	30	1–4
1985	Vertical	80	9	26	1–6
1986	BIONESS	333	150	35	1–4
1986	Vertical	80	9	35	5–7

small zooplankton size categories. The biomasses of all zooplankton size fractions were transformed to natural logarithms prior to analysis to eliminate the positive correlation between mean and variance in the untransformed data. Zooplankton biomass distributions were graphed with Golden Graphics System software (Golden Software, Inc., Golden, CO). The program creates a regularly spaced grid from irregularly spaced data using the regional variable theory technique called Kriging. The Kriging algorithm assumes an underlying linear variogram (Ripley 1981).

Results and Discussion

Coincident spatial distributions were evident in the biomass distributions of equivalent zooplankton size classes sampled with the two gear types. In both years, the total biomass (milligrams per cubic metre) of zooplankton >308 μm retained by the 333-μm BIONESS was positively correlated with the biomass of this size class collected with the 80-μm vertical net (1985: $r = 0.55$, $n = 22$, $p < 0.01$; 1986: $r = 0.36$, $n = 35$, $p < 0.05$). The biomass of zooplankton >308 μm collected in the vertical hauls was consistently two to five times greater at each station relative to the mean biomass of this size class sampled with the BIONESS. The average biomass of zooplankton in the >308 μm size class over the full survey area was 131 and 22 mg/m³ for the vertical haul and BIONESS samples, respectively, in 1985 and 73 versus 40 mg/m³ in 1986. The lower biomass of zooplankton >308 μm collected by the BIONESS may have resulted from undersampling certain parts of the water column relative to the vertical net and/or poor retention of the smaller zooplankton by the 333-μm-mesh net.

There was a positive correlation between the biomass of the >308 μm size class collected with the BIONESS and distance from shore in both years (Fig. 2) (1985: $r = 0.61$, $n = 30$, $p < 0.01$; 1986: $r = 0.36$, $n = 35$, $p < 0.05$). An identical analysis of the vertical net collections yielded a similar result (1985: $r = 0.41$, $n = 26$, $p < 0.05$; 1986: $r = 0.30$, $n = 35$, $p < 0.10$).

Several authors have assumed that the biomass retained by the coarse-mesh net provides a reliable index of food available to fish larvae. If true, a positive correlation should exist between the biomass of small (<308 μm) plankters and distance from shore in the survey area. I used the biomass estimates of zooplankton <308 μm retained by the vertical net to evaluate the assumption. Of particular interest was the distribution of the 80–153 μm size class that includes copepod eggs and nauplii (size range = 100–150 μm) which comprise 70–80% of the diet of cod and haddock larvae in the size range 3.5–7.0 mm (Kane 1984; Laurence and Lough 1985). The assumption was not supported. The biomass of particles 80–153 μm was negatively correlated with distance from shore in both years (1985: $r = -0.50$, $n = 26$, $p < 0.05$; 1986: $r = -0.66$, $n = 35$, $p < 0.001$) and highly significant inverse correlations existed between the biomass of zooplankton in the >308 μm size class as estimated with the BIONESS and the biomass of zooplankton 80–153 μm sampled with the vertical net (Fig. 3) (1985: $r = -0.55$, $n = 22$, $p < 0.01$; 1986: $r = -0.47$, $n = 35$, $p < 0.01$; 1985 + 1986: $r = -0.61$, $n = 57$, $p < 0.001$). The concentration of 80–153 μm zooplankton was greatest in the nearshore and declined rapidly with distance offshore (Fig. 4).

Analysis of the full range of particle size classes obtained with the two gear types in both 1985 and 1986 showed that the biomasses of adjacent particle size classes were strongly positively correlated, more distant size classes were weakly positively correlated, and the extremes of the range of size categories were inversely correlated (Table 2). In addition, I analyzed the full range of particle size classes obtained with the 80-μm vertical net only (Table 3) and found a result nearly identical to the two gear type analysis (Table 2).

Spatial variation in zooplankton size composition was assessed from the vertical samples collected during 1985. The 1986 samples could not be assessed because only three size fractions were processed (Table 1). The weighted mean particle size (calculated by weighting the mesh size of the six sieves used to fractionate the plankton by the biomass on each sieve) was generally <400 μm at stations <60 km from shore and exceeded 900 μm at most stations >80 km from shore (Fig. 5). A significant positive correlation was evident between mean particle size and distance from shore ($r = 0.63$, $p < 0.01$). This result mirrored the reciprocal biomass distributions of large (>308 μm) and small (80–153 μm) zooplankton evident during both years of the survey.

The strength of the correlations between adjacent particle size classes and between large and small zooplankton may vary on a seasonal basis. Zooplankton data collected with 80-μm-mesh nets towed vertically and 333-μm-mesh bongo nets towed obliquely from 30 m to the surface on three research cruises to the southwestern Scotian Shelf (Browns Bank) were used to assess this possibility. Sampling periods were 23–30 May, 12–16 July, and 14–21 November 1983. Additional details of the sampling program are contained in Frank (1986). The correlation matrices generated from the May and November size-fractionated zooplankton biomass data were similar (Table 4) and identical to the results obtained in May of 1985 and 1986 (Table 2). A different pattern existed in July when the smallest size fraction (80–308 μm) was positively correlated with the larger, nonadjacent size fractions whereas adjacent size classes were often weakly correlated (Table 4). July distributions also differed from those of May and November by exhibiting relatively high positive correlations between large (>308 μm) and small (<308 μm) zooplankton biomass (Fig. 6A) whereas a weak negative correlation or no correlation occurred between large and small particle biomass in May and November of 1983, respectively (Fig. 6A).

I analyzed the zooplankton biomass data in Lambert et al. (1982) and found that the strongest positive correlation between large (>253 μm) and small (<253 μm) particle biomass during the interval June–December 1984 in St. Georges Bay occurred during July (Fig. 6B). Hargrave et al. (1985) attributed this high, positive correlation between large and small particles during July in St. Georges Bay to synchronous copepod reproduction. This interpretation may also apply to the July data from Browns Bank. Seasonal changes in the size and species composition of the zooplankton community linked to variation in water temperatures (Deevey 1960; Ware 1977) provide another possible explanation.

The inverse spatial patterns of abundance of large and small zooplankton (Fig. 2, 4) are consistent with findings in several other areas (Table 5). While the number of published studies is limited, independent spatial distributions of different developmental stages of several copepod species and of discrete size groups of zooplankton are common results. Williams et al. (1983) have noted that differences in depth distributions of various copepod developmental stages could result in separate spatial distributions. Behavioural as well as passive factors could contribute to this vertical separation. For instance, *Calanus fin-*

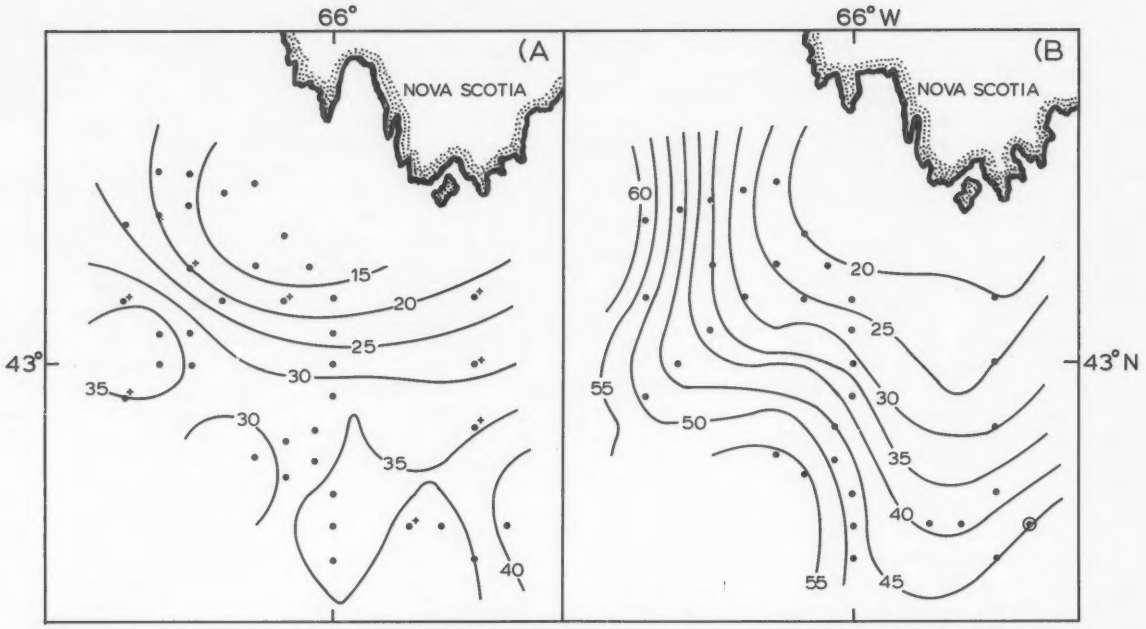

FIG. 2. Distribution (mg dry wt/m³) of zooplankton biomass >308 μm averaged over the water column from BIONESS collections made during (A) May 1985 and (B) May 1986. A plus sign (+) in Fig. 2A indicates stations (●) where zooplankton biomass (Y) was predicted from distance (X, km) using the equation $\ln Y = (1.02) \ln Y - 1.33$. The circled station in Fig. 2B was sampled twice 10 d apart.

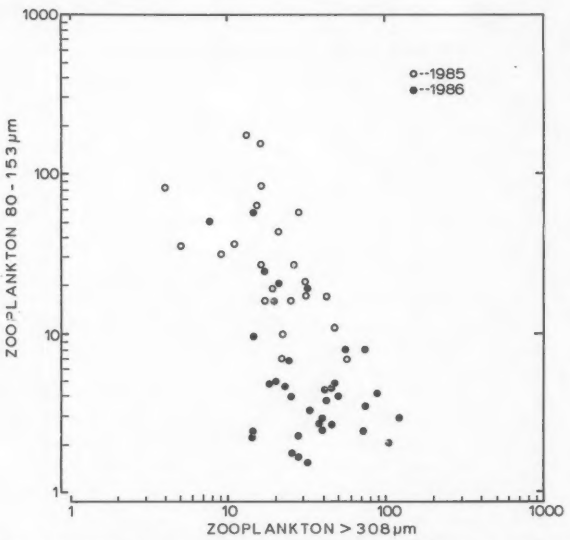

FIG. 3. Plot of the biomass of zooplankton in the >308 μm size class collected with the BIONESS and the biomass of zooplankton 80–153 μm sampled with the vertical net from 1985 and 1986.

marchicus eggs are denser than seawater, sink at a rate of about 2.5 cm/min at 13°C, and if laid at the surface the eggs will hatch at a depth of 36 m approximately 24 h later (Marshall and Orr 1955).

The higher concentrations of small zooplankton in nearshore waters (biomass: Fig. 4; particle size composition: Fig. 5) are consistent with the findings of several earlier studies (Table 6) in which fine-mesh nets (<158 μm) were used to evaluate mesoscale zooplankton distributions. Collectively, these studies show that cross-shelf particle size gradients involving small zooplankton (i.e. copepod eggs, nauplii, small copepod species) are common and that these smaller, edible plankton are more abundant in nearshore or coastal waters than in continental shelf or slope waters. Interestingly, all of the studies listed in Table 6 that used coarse-mesh nets (>253 μm) (with the exception of Loeb et al. 1983) showed the inverse pattern. This artifact appears to be created by the proportionately greater loss of small zooplankton through the net meshes at the coastal locations. Variation in efficiency of sampling from area to area biases among-area comparisons (Green 1979).

Independence of Fish Larvae and Prey Distributions — A Reassessment

The finding that zooplankton samples collected with large-mesh nets do not provide a reliable qualitative or quantitative estimate of the abundance of prey available to larval fish demands that previously published studies (Bay of Fundy – Gulf of Maine: Sinclair and Iles 1985; Scotian Shelf: O'Boyle et al. 1984; California Current: Sinclair et al. 1985; Grand Bank: Anderson and Gardner 1986) claiming the existence of independent distributions of fish larvae and prey be reassessed.

Sinclair and Iles (1985) showed that the highest larval abundances of several groups of fall spawning Gulf of Maine – Bay of Fundy herring occurred in areas having relatively low summer and autumn zooplankton biomass. They concluded that the larval distributional areas are not rich in terms of larval food relative to adjacent areas outside of the larval concentrations. This conclusion was based on total (=unsorted) zooplankton displacement volumes determined from collections made during August and November 1981. A 0.5-m-diameter, 253-μm-

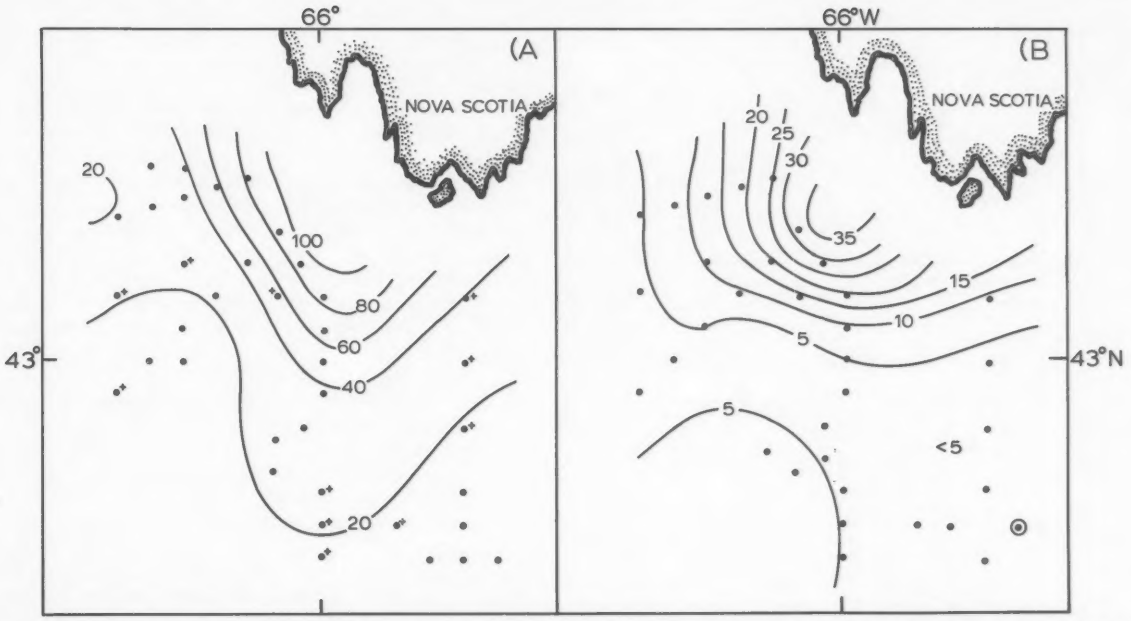

FIG. 4. Distribution (mg dry wt/m³) of 80–153 μm size category of zooplankton biomass averaged over the water column from vertical haul collections made during (A) May 1985 and (B) May 1986. A plus sign (+) in Fig. 4A indicates stations (●) where zooplankton biomass (Y) was predicted from distance (X, km) using the equation $\ln Y = 7.58 - (1.003) \ln X$. The circled station in Fig. 4B was sampled twice 10 d apart.

TABLE 2. Correlation matrix derived from the analysis of the full range of zooplankton biomass size categories (μm). Data originated from BIONESS (BB) and vertical haul (VH) collections made in the waters off southwestern Nova Scotia. Top right half and bottom left half of the panel show 1985 and 1986 results, respectively. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	>1050, BB	860–1050, BB	602–860, BB	308–602, BB	153–308, VH	80–153, VH
>1050, BB	—	0.968***	0.660***	0.177	−0.031	−0.660***
860–1050, BB	0.919***	—	0.721***	0.274	−0.070	−0.662***
602–860, BB	0.632***	0.831***	—	0.770***	0.013	−0.217
308–602, BB	0.028	0.272	0.679***	—	−0.021	0.144
153–308, VH	−0.277	−0.289	−0.185	0.099	—	0.298
80–153, VH	−0.581***	−0.577***	−0.345*	0.242	0.471**	—

TABLE 3. Correlation matrix derived from the analysis of the full range of zooplankton biomass size categories (μm) originating from the vertical haul collections made in the waters off southwestern Nova Scotia during 1985. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

	860–1050	602–860	308–602	153–308	80–153
>1050	0.94***	0.74***	0.61***	0.42*	−0.40*
860–1050		0.86***	0.72***	0.51**	−0.21
602–860			0.89***	0.62***	0.16
308–602				0.85***	0.22
153–308					0.30

mesh ring net hauled vertically from near bottom (+3 m) to the surface was used to collect all zooplankton samples (M. Power, Department of Fisheries and Oceans, St. Andrew's, N.B., pers. comm.). Approximately 150 stations, uniformly distributed throughout the Bay of Fundy and its approaches, were sampled. Zooplankton displacement volumes outside the principal larval distributional areas exceeded those recorded inside these areas by 2.5 and 5.4 times in August and November, respectively (see fig. 5 in Sinclair and Iles 1985). The prey types recorded in the diet of young (<4 wk post-hatching) herring larvae collected at sea include a variety of zooplankton, dominated by copepod eggs, nauplii, and copepodites in the 100–200 μm size range (Blaxter 1965; Bjorke 1978; Cohen and Lough 1983). Consequently, the relevant prey biomass for young herring larvae in the Bay of Fundy area was not directly measured. Sinclair and Iles (1985) used the displacement volume of zooplankton >253 μm to map the distribution of larval food. However, Sameoto (1977) reported zooplankton distributions in the southwestern Nova Scotia region during August 1974 that were markedly different from those portrayed in Sin-

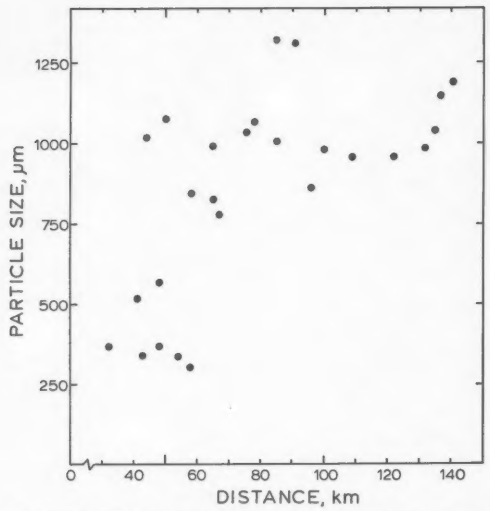

FIG. 5. Spatial trend in zooplankton size composition in relation to distance offshore. Weighted mean particle sizes were calculated from 1985 vertical haul samples.

clair and Iles (1985) even though the gear types, mesh sizes, and deployment schemes used were similar. Highlighted briefly, Sameoto's data showed that copepod biomass in the nearshore, shallow area off southwestern Nova Scotia, an area identified by Sinclair and Iles (1985) as a major larval retention zone, was 1.5-fold greater (mean = 171 mg wet wt./m³, range = 89–372) than the biomass measured at adjacent stations in deeper water outside the retention area (mean = 117 mg/m³, range = 25–178). *Pseudocalanus* sp., a preferred prey item of young herring larvae (Checkley 1982), was 2.2 times more abundant at stations inside the retention area (mean = 1630 individuals/m³) relative to stations outside (mean = 744/m³).

Two important sources of bias are inherent in the analysis of zooplankton distributions reported by Sinclair and Iles (1985). First, macrozooplankton were not sorted from the remainder of the sample prior to displacement volume determinations, and second, zooplankton displacement volume was expressed as millilitres per haul without correcting for differences in the volume filtered which resulted from different water depths between stations. It is impossible to evaluate the magnitude of the bias caused by the first problem. The bias introduced by failure to correct for different sampling depths can, however, be assessed. The average depth of stations inside and outside the Bay of Fundy larval herring retention zones identified by Sinclair and Iles (1985) was 62 and 119 m, respectively. I found a significant positive correlation between zooplankton displacement volume (millilitres) and station depth in both the August 1981 ($r = 0.44$, $p < 0.001$) and November 1981 ($r = 0.51$, $p < 0.001$) surveys. Zooplankton displacement volume at each station corrected for volume filtered (millilitres per cubic metre) was negatively correlated with station depth during both August ($r = -0.63$, $p < 0.001$) and November ($r = -0.14$, $0.05 < p < 0.10$), a result roughly consistent with the copepod biomass data presented by Sameoto (1977). The volume-corrected data alone contradict the conclusion of Sinclair and Iles (1985) and would suggest that the larval herring retention zones were relatively rich in larval food. Given the fact that the retention zones of this area are located in coastal areas where the highest concentrations of smaller, edible zooplankton occur (Fig. 4; Table 6), the opposite conclusion may indeed be true. Clearly, further study of the zooplankton dynamics within this region is required.

O'Boyle et al. (1984) analyzed zooplankton data collected in 1979 during the Scotian Shelf Ichthyoplankton Program (SSIP). Their analysis showed that the larval food supply on the offshore banks was occasionally higher than that in the contiguous waters. Larval food supply was estimated from 0.61-cm-diameter, 333-µm-mesh bongo net hauls towed obliquely through the entire water column. Significant inverse correlations between station depth and total numbers per cubic metre of zooplankton >333 µm (log transformed) were observed in the April ($R^2 = 0.03$, $n = 115$), May ($R^2 = 0.31$, $n = 75$), September–December ($R^2 = 0.15$, $n = 89$), and November–December ($R^2 = 0.41$, $n = 117$) surveys. O'Boyle et al. (1984) concluded that zooplankton prey for larval fishes seemed to be more concentrated on the relatively shallow (<100 m depth) offshore banks, especially during spring and early summer.

TABLE 4. Correlation matrix derived from the analysis of zooplankton biomass size categories estimated from 333-µm-mesh bongo (B) and 80-µm vertical (VH) net collections made during May ($n = 22$), July ($n = 8$), and November ($n = 7$) of 1983 on Browns Bank. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

	>1050, B	860–1050, B	602–860, B	308–602,B
May				
860–1050, B	0.740***			
602–860, B	0.586**	0.820***		
308–602, B	0.172	0.179	0.454*	
80–308, VH	0.256	0.025	0.408	0.518
July				
860–1050, B	0.334			
602–860, B	−0.106	0.786*		
308–602, B	−0.099	0.519	0.790*	
80–308, VH	0.368	0.729*	0.488	0.128
November				
860–1050, B	0.908***			
602–860, B	0.607	0.713		
308–602, B	0.543	0.621	0.625	
80–308, VH	−0.022	−0.096	−0.068	0.654

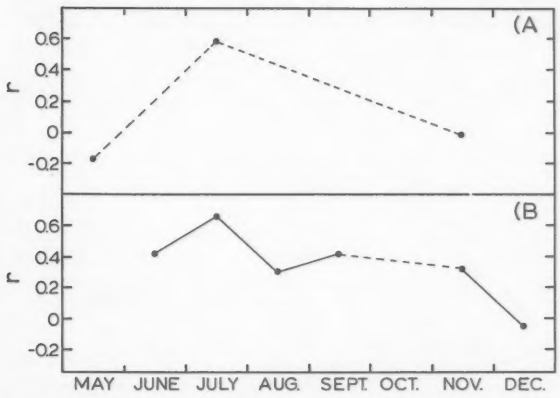

FIG. 6. Monthly correlation coefficients between large and small zooplankton from (A) Browns Bank (80–308 μm vs. zooplankton biomass >308 μm) during 1983 and (B) St. Georges Bay (80–253 μm vs. zooplankton biomass >253 μm) during 1974.

Their finding that large (>333 μm) zooplankton were more abundant offshore is entirely consistent with my 1985 and 1986 zooplankton biomass data from the Browns Bank region (Fig. 2). The major elements of the ichthyoplankton considered by O'Boyle et al. (1984) included cod, haddock, pollock, and silver hake. The early feeding stages of two of these species require prey in the 100–150 μm size range (Kane 1984) which are not sampled by the 333-μm-mesh nets. Moreover, as my study and those in Table 5 have shown, the biomasses of larger zooplankton are generally inversely correlated with those of smaller zooplankton known to be the principal prey of larval fish. In addition, systematic collections made with coarse-mesh nets often lead to inaccurate descriptions of the seasonal cycle of zooplankton abundance. This was demonstrated by Deevey (1956) in Long Island Sound where the maxima and minima of the annual distributions of weekly zooplankton volumes measured by two nets of different mesh (153 versus 366 μm) often did not coincide. Maxima in late spring and late summer were evident in the 153-μm-mesh net collections compared with April and September maxima resulting from the 366-μm-mesh net samples. I conclude that the zooplankton data used by O'Boyle et al. (1984) did not adequately reflect the temporal or spatial characteristics of the food supply for the young stages of larval gadoids of the Scotian Shelf waters.

Anderson and Gardner (1986) reported ichthyoplankton distributions on the Southeast Shoal region of the Grand Bank that were noticeably distinct from the high abundance of zooplankton that would normally constitute their prey. In fact, significant inverse correlations were evident between ichthyoplankton abundance and three interdependent measures of zooplankton abundance (displacement volume: $r = -0.70$; *Calanus finmarchicus* density: $r = -0.61$; *Pseudocalanus* sp. density: $r = -0.72$). The authors acknowledged that the main dietary components of small fish larvae (copepod eggs, nauplii, small copepodites) were not quantitatively sampled by the 333-μm-

TABLE 5. Studies showing independent spatial distributions of (a) copepod eggs and nauplii versus adult and copepodite stages and (b) large versus small zooplankton size classes.

	Region	Sampling protocol	Type of analysis	Reference
(a) Copedod species				
Calanus pacificus	Nearshore California Current, off southern California	200-m contiguous pump sampler from fixed depth (34 m) along 30 km transect	Cluster analysis	Star and Mullin 1981
Calanus helgolandicus	Western Mediterranean sea, Ligurian Sea	1.2-km contiguous pump sampler from fixed depth (14 m) along 43-km transect	Principal components analysis	Boucher 1984
Acartia tonsa *Temora longicornis* *Calanus finmarchicus*	Western North Sea, Buchan area	Depth-integrated samples from 0–70 m using submersible pump; 5-nm station spacing along two 60-nm transects	Comparison of density distributions; general agreement between transects	Kiørboe and Johansen 1986
Calanus marshallae	Pacific Ocean, Oregon coastal upwelling zone	Discrete depth sampler to near bottom or 100 m using both Clarke–Bumpus and pump along four 10–30 km cross-shelf transects; station spacing ~5 km	Comparison of density distributions; general agreements between transects	Peterson et al. 1979
(b) Size group				
Small (0.2–0.9 mm) vs. large (>1.0 mm)	Nearshore and offshore waters off southern California	Pump towed 1.6 km at 5 m depth at 162 stations over 6000-mi² area	Correlation	O'Connell 1971
Biomass size groups: 100–500 μm vs. zooplankton >500 μm	Upwelling area off Spanish Sahara	Weekly vertical net hauls from 200 m or bottom using bongos at 5 cross-shelf stations spaced a: 10 km from March to May	Comparison of biomass size group distribution; persistent pattern evident among sessions	Blackburn and Nellen 1976

TABLE 6. Evidence from field studies showing differential abundance or biomass between nearshore and offshore waters.

Reference	Location	Sampling methodology	Conclusions
Arthur 1977	California Current system	Vertical net haul with 76-μm mesh	3 times as many copepods ≤1 mm and 12 times as many nauplii on average throughout onshore as in the offshore zone
Beers and Stewart 1970	California Current off La Jolla	Pumping system with deck-mounted filtration by 35-μm-mesh cloth	At 3 stations located 1.4, 4.6, and 12.1 km from shore, naupliar densities averaged 63, 33, and 26/L, respectively
Deevey 1956	Long Island Sound, Block Island Sound, Georges Bank	Clarke–Bumpus fitted with No. 10 (158 μm) silk net towed obliquely	10–20 times increase in total numbers of zooplankton in passing from offshore to inshore and then to more enclosed, less saline waters. Accompanying this was large decrease in mean size of individuals because mean displacement volumes/m³ did not vary widely (1–3 times)
St. John 1958	Cape Hatteras – Florida Current	Clarke–Bumpus fitted with No. 2 (~360 μm) silk net towed obliquely	Volumes of zooplankton least in shallow coastal areas and in offshore Florida Current and maximal near midshelf region
Sinclair and Iles 1985	Gulf of Maine – Bay of Fundy	Vertical haul of standard plankton net (253 μm) mesh	Generally low displacement volumes of zooplankton associated with shallow coastal waters compared with deeper offshore waters (3–5 times difference on average)
Peterson 1972	Northeast Pacific off Washington and Oregon coast	Clarke–Bumpus fitted with 110-μm-mesh net towed horizontally at different depths down to 100 m	Abundance and production of copepod nauplii greater on shelf than in oceanic or slope waters; ratio of copepod nauplii to total copepods 2–4 times greater nearshore than offshore with greater volume of zooplankton farther offshore
Peterson et al. 1979	Oregon coastal upwelling zone	Same as above except 120-μm-mesh net	Copepod eggs, nauplii, and early copepodite stages decline in average abundance with increasing distance offshore; highest average abundance of adults occurs at an intermediate distance
Clarke 1940	New York Bight	Oblique hauls of 1.5-m-diameter, 158-μm-mesh net	Progressive decline in combined abundance of *Calanus* sp. and *Centropages* sp. from coastal to slope waters; 1–2 orders of magnitude difference
Smith 1971	California Current	550- and 471-μm-mesh CalCOFI net towed obliquely	Zooplankton displacement volume was maximum at 80–120 miles offshore and diminished toward the coast; offshore–onshore trend of higher standing stocks offshore which diminish seaward and inshore was typical along entire coast.
Loeb et al. 1983	California Current	505-μm-mesh CalCOFI net towed obliquely	Within the 1975 survey area, zooplankton displacement volumes decreased from north to south and from inshore to offshore

mesh net used in their study. They assumed, however, that the distribution of the larger copepods was an accurate reflection of the larval prey distributions. The authors considered the reciprocal larval and zooplankton abundances "paradoxical" and they questioned not their assumption that the coarse-mesh net provided a reliable estimate of prey biomass but rather the source of food production for fish larvae on the Grand Bank. Independent spatial distributions of immature and mature copepods appear to be the rule (Table 5) and provide a simple explanation for the apparent "paradox" in their data.

Sinclair et al. (1985) reported higher survival rates to age 1 for Pacific mackerel during years of relatively low zooplankton biomass in the California Current. Periods of low zooplankton biomass were associated with decreased southward transport inferred from sea level variation. The authors concluded that survival rates during the early life history of Pacific mackerel may be influenced more by direct hydrographic processes than by biological interactions. The average zooplankton biomass values used in their analysis were estimated by sampling the upper 140 m of the water column with a CalCOFI 550-μm-mesh net deployed in the offshore, southern California Bight region (area = 30 000 km²) during March, June, September, and December 1951–65. During the same period, zooplankton was sampled at 28 stations in the upper 15 m of a coastal embayment off Santa Monica with a 64-μm-mesh net (Haydock 1973). Annual means of zooplankton biomass in the >64 μm size class were found to be positively correlated ($p < 0.01$) with the biomass of zooplankton in the >550 μm size class in each of the four months sampled ($n = 15$ yr). Sinclair et al. (1985) inferred from this relationship that the zooplankton biomass estimated with the coarse-mesh net was a reliable index of larval food. However, in the Haydock data the biomass of prey in the size range edible by larval mackerel (e.g. larvae 4–10 mm ingest prey, on average, 90–235 μm in width, Hunter and Kimbrell 1980) was not isolated from the larger, inedible component of the sample. It is therefore impossible to assess the extent to which even the Haydock samples reflect the abundance of edible plankton available to larval fish.

Beers and Stewart (1967, 1969) demonstrated that microzooplankton volumes (103–202 μm) collected by pump sampling represented between 10 and 30% of the larger (>550 μm) zooplankton volumes estimated from the CalCOFI data. There was also a general decrease in the abundance of the <202 μm zooplankton from coastal to oceanic stations. This pattern is consistent with the result of other quantitative zooplankton studies undertaken in the California Current and waters to the north off Oregon and Washington (Table 6). The only apparent exception to this pattern was reported by Smith (1971), but his data were derived from coarse-mesh (>550 μm) samples and were expressed as displacement volumes.

It is also known that the quantity and size composition of plankton in the California Current vary from year to year in a complex way. Lasker (1981) reported that anchovy year class strength during the years 1962–77 was unrelated to total plankton production but showed some relationship to the size of the dominant plankter. During years of reduced large-scale horizontal advection of cold, nutrient-rich water from the north, sea surface temperatures are relatively high and zooplankton displacement volumes are low. The opposite holds when horizontal advection is high (Reid et al. 1958; Reid 1962). Both Deevey (1956) and Ware (1977) have documented inverse relationships between water temperature and the mean size of marine zooplankton. Temperature-induced interannual variation in the average particle size of zooplankton in the California Current could cause differential retention of material by the 550-μm-mesh nets used in the CalCOFI program. This could contribute to the variable displacement volumes observed.

The California Current system is also characterized by localized upwelling events that result in high interstation variability in zooplankton biomass (Smith 1978; Loeb et al. 1983). Seasonal indices of dispersion ($s^2/\bar{X}$) for zooplankton biomass can vary greatly in the central sector of the California Current in some years (e.g. 1957: range = 25–1840) and only moderately in others (Haury et al. 1978). Analysis of tow-to-tow sample variability within 11 regions of the CalCOFI survey area during 1975 showed frequent cases of high variation within and between cruises throughout all regions and in all years. This variation was especially pronounced in the northern coastal region of the survey area (Loeb et al. 1983). Spatial averaging such as performed on the data used by Sinclair et al. (1985) masks most of the heterogeneity in the system and makes the demonstration of statistically significant differences in zooplankton abundance within and among regions difficult.

In summary, the influence of artificial effects, such as the comparison of biomass data from widely separated geographic locations using contrasting sampling methodologies and the inconsistent averaging of time and space intervals, has distorted the true biological patterns characteristic of the California Current system. In light of the preceding arguments, alternative hypotheses concerning the high survival rates to age 1 of Pacific mackerel during years of low zooplankton biomass >550 μm should be devised and tested.

Conclusion

The importance of defining the target population to be studied and the sampling requirements necessary to quantify its dynamics cannot be overstated. Green (1979) reminds us that no area of environmental studies is immune to the problem of inefficiency and bias in sampling methods and he recommended that the sampling device or method selected to quantify the target population be verified to ensure equal and adequate efficiency over the entire range of sampling conditions to be encountered. It is apparent from the new work presented here and from the reanalysis of previously published work that the reciprocal distributions of fish larvae and their prey recently reported in the literature have been seriously biased by the collection methods used to quantify the larval food resource. The evidence is clear: coarse-mesh net samples *do not* provide an accurate reflection of the distribution of food for larval fish. Coarse-mesh nets not only fail to sample much of the zooplankton present but, they also fail to provide reliable estimates of temporal trends in net plankton distribution (Deevey 1956).

On evolutionary grounds it is reasonable to expect that areas where larval fish are typically distributed will be coincident with high food resources. This is supported by the observation that the larval stage of most species is characterized by rapid growth relative to the other phases in the life history. Larval fish distributions that deviate from this expectation may be symptomatic of the influence of an environmental factor important in regulating population abundance. However, before this conclusion is drawn it must be clearly shown that biases have not been introduced into the evaluation of the larval food resource by the sampling procedures employed. The apparent anomalous persistence of herring larvae in geographically predictable coastal locations in the Bay of Fundy area "in spite of the particular plankton characteristics" (Sinclair and Iles 1985) is a case in point. My reanalysis of the zooplankton data presented in Sinclair and Iles (1985), the new data presented here, and the reports of previous (Sameoto 1977) zooplankton studies conducted in the region all suggest that these larvae remain in the area *because* of rather than *in spite of* the food resources available. In this light the retention zone hypothesis proposed by Sinclair and Iles (1985) to explain the "apparent paradox" of the zooplankton prey for larval fish must be seen as support for the match/mismatch hypothesis it was designed to replace.

In some situations, temporary independent spatial distributions of fish larvae and their prey may occur. However, these appear to be temporary and restricted to the yolk-sac stage (Fortier and Leggett 1984; Taggart and Leggett 1987). Subsequent correspondence in the distributions may evolve as a result of food-mediated differential mortality or as a result of kinetic responses of larvae towards regions of high prey concentration (Frank and Leggett 1983; Fortier and Leggett 1984).

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Effects of Constant Day Length on Sexual Maturation and Growth of Atlantic Salmon (*Salmo salar*) Parr

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Saunders, R. L., and E. B. Henderson. 1988. Effects of constant day length on sexual maturation and growth of Atlantic salmon (*Salmo salar*) parr. Can. J. Fish. Aquat. Sci. 45: 60–64.

Atlantic salmon (*Salmo salar*) were reared for 6 mo from first feeding under four photoperiod regimes: LD 12:12, LD 16:8, LD 24:0, and simulated natural (LDN). Water temperature followed seasonal changes but was adjusted so as not to exceed 16°C in summer or to fall below 6°C in winter. Growth rates were highest in the LD 24:0 group during the first 3 mo; subsequently, the LD 16:8 fish grew faster than all others. Length–frequency distributions were skewed towards smaller sized fish in January. Sexually mature males were mainly in the lower halves of the length–frequency distributions; females and immature males were distributed throughout. The incidence of mature males was highest in the LDN group (67% of males) but reached high levels (44–58%) in the other groups.

Des saumons de l'Atlantique (*Salmo Salac*) ont été élevés pendant 10 mois à partir du moment où ils ont commencé à se nourrir sous quatre photopériodes : LO (lumière:obscurité) 12:12, LO 16:8, LO 24:0 et LO naturelles (LON). La température de l'eau était conforme aux variations saisonnières, mais ajustées de façon à ne pas excéder 16°C pendant l'été ou descendre en bas de 6°C pendant l'hiver. Pendant les trois premiers mois, les taux de croissance étaient plus élevés dans le groupe LO 24:0; par la suite, les poissons du groupe LO 16:8 ont crû plus vite que tous les autres. En janvier, les distributions de fréquences des longueurs étaient asymétriques, les petits poissons étant plus nombreux. Les mâles ayant atteint la maturité sexuelle se trouvaient principalement dans la moitié inférieure des courbes de distribution de fréquences de longueurs; les femelles et les mâles immatures étaient distribués dans tous les classes de taille. La fréquence de mâles matures la plus élevée a été observée pour le groupe LON (67 %) des mâles), tout en restant éleyée (44–58 %) dans les autres groupes.

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Artificially increased day length applied during the appropriate season and during certain developmental stages may be used to enhance growth rates of salmonids (Saunders and Henderson 1970; Komourdjian et al. 1976; Lundqvist 1980; Brauer 1982; Saunders et al. 1985). Enhancement of growth of juvenile Atlantic salmon (*Salmo salar*) is practiced in rearing establishments to maximize efficiency through production of 1+-yr-old smolts. Thorpe (1977), Thorpe et al. (1980), Bailey et al. (1980), Thorpe et al. (1982), and Kristinsson et al. (1985) have demonstrated that juvenile Atlantic salmon develop bimodal length–frequency distributions during their first autumn. The upper and lower modes represent the potential 1+ and 2+ smolts, respectively. Females and immature males are found in both modes; most sexually mature males, because of reduced growth rates during maturation (Bailey et al. 1980; Lundqvist 1980; Thorpe and Morgan 1980; Kristinsson et al. 1985), are in the lower mode. Manipulation of photoperiod can influence the proportions of parr in the upper and lower modes and, therefore, the proportion of fish which become 1+ smolts (Kristinsson et al. 1985). Saunders et al. (1982) suggested that the incidence of underyearling, mature male parr can be regulated by adjusting the time of first feeding and growth rates during the first summer. It is desirable to minimize the incidence of mature male parr and to have more of the population in the upper length–frequency mode.

In this paper we report the effects of three constant day-length regimes and simulated natural photoperiod on the incidence of sexual maturity in underyearling male Atlantic salmon parr in relation to growth and length–frequency distribution.

Materials and Methods

Atlantic salmon of Magaguadavic River (N.B.) stock were incubated at the North American Salmon Research Center, St. Andrews, N.B. On May 23, 10 000 alevins were transferred to the St. Andrews Biological Station and distributed equally among eight 1-m² Swedish-style rearing tanks supplied with running freshwater. On May 24, commensurate with swim-up and first feeding, four photoperiod regimes were initiated: 12 h light : 12 h dark (LD 12:12), 16 h light : 8 h dark (LD 16:8), 24 h light (LD 24:0), and simulated natural (LDN) for latitude 45°N. Tanks were illuminated using a pair of 40-W daylight fluorescent lamps suspended 1 m above the water surface, giving light intensities at the water surface of 430–540 lx. Photoperiod was controlled by electric timers; lights were switched on and off without simulated dawn and dusk periods. There were initially two groups under each of the four photoperiod treatments. On December 21, one of the LDN groups was lost owing to a clogged water supply line. No significant difference in fish performance between replicates was detected. There-

fore, although three of the groups were replicated, data from these replicates were pooled with their respective treatment groups. To prevent overcrowding, the number of fry was reduced to 650 on June 20 and to 450 on August 30 by random removal of excess fish.

Temperature was regulated in accordance with natural seasonal trends but was adjusted so as not to exceed 16°C in summer or to fall below 6°C in winter. Temperatures among tanks varied less than ±0.5°. Running freshwater was supplied at increasing rates as the biomass increased to maintain dissolved oxygen levels at >75% of air saturation.

All groups of fish were fed to satiation on commerical salmon food (Ewos or Silver Cup) using automatic feeders operating during daylight hours; the length of feeding time corresponded with the group receiving the shortest photoperiod, LD 12:12 during spring and summer and LDN during fall and winter. Although the fish were rarely observed to take uneaten food from the bottoms of rearing tanks, those reared under greater day length may have done so during periods when fresh food was not offered.

Measurements of fork length (nearest 0.1 cm) and weight (nearest 0.1 g) were made on August 28 and January 2 based on random samples of each group. The August sample was approximately 100 fish per treatment group; the January sample was approximately 200 fish for the LD 12:12, LD 16:8, and LD 24:0 photoperiod treatments and 100 for the LDN group. The fish were starved for 24 h before sampling and anaethetized with 1–3% tertiary amyl alcohol to facilitate handling. On January 2, the sampled fish were killed and necropsied to determine sex and state of sexual maturation. Males with fully developed or developing testes were counted as mature; mature or maturing testes had a creamy white appearance and were clearly distinguishable from immature testes which were pink and threadlike.

One-way ANOVA and Duncan's multiple range test were used for statistical tests of differences in mean length and weight among the four day length regimes on August 28. Two-way ANOVA was used for the January 2 data to determine if there were (1) significant differences in mean length and weight among day length regimes, (2) differences in mean length and weight between sexes of the pooled treatments, and (3) interaction between day length regimes and sex. One-way ANOVA was then used to compare mean length and weight between day-length regimes for each sex. Statistical tests of differences among incidence of sexually mature male parr in response to photoperiod treatment were conducted using the normal approximation of the binomial (Brownlee 1960).

Results

Juvenile Atlantic salmon reared under continuous light (LD 24:0) from first feeding on May 24 were significantly longer and heavier ($p < 0.05$) by August 28 than those reared under LD 12:12, LD 16:8, and LDN (Fig. 1). The LDN fish were significantly longer ($p < 0.05$) then the LD 12:12 and LD 16:8 fish and significantly heavier than the LD 16:8 fish. By early January, the LD 16:8 fish were significantly heavier and longer ($p < 0.05$) than all other groups (Fig. 1). Two-way ANOVA showed a significant interaction between photoperiod regime and sex ($p < 0.001$). Immature males and females from the LD 16:8 regime were longer and heavier than fish from the other regimes, although in some instances the differences were not significant. Mature males from the LD 12:12 treatment were significantly longer and heavier ($p < 0.05$) than those from LD 16:8 and LDN (Fig. 1). Length–frequency distributions were skewed towards the smaller sized fish in early January; there were slight indications of bimodality (Fig. 2).

There was a high incidence of sexually mature or maturing male parr under each photoperiod treatment (Table 1). No mature or maturing females were observed. A significantly greater number of males matured under the LDN regime than under LD 16:8 ($p < 0.05$). The incidence of mature males under the LDN regime was not significantly greater than under LD 12:12 and LD 24:0. Sexually mature males were confined, almost entirely, to the lower halfs of the length–frequency distributions (Fig. 2). Females and immature males were represented throughout the frequency distributions.

Discussion

Higher growth rates have been reported for juvenile Atlantic salmon exposed to increased day length in comparison with those under simulated natural photoperiod (Saunders and Henderson 1970; Komourdjian et al. 1976; Lundqvist 1980; Kristinsson et al. 1985; Saunders et al. 1985). The results of the present study were in general agreement with these earlier findings; the LD 12:12, LD 16:8, and LD 24:0 fish were significantly larger in January than those in the LDN group (Fig. 1). However, the smaller size of the fish in the LDN group probably resulted in part from the high incidence of mature males (Table 1). A number of workers have reported that growth rate of male Atlantic salmon parr decreases during late summer – autumn as they mature (Saunders and Sreedharan 1977; Eriksson et al. 1979; Bailey et al. 1980; Lundqvist 1980; Thorpe et al. 1982; Kristinsson et al. 1985). Thorpe et al. (1980, 1983) and Saunders et al. (1982) reported that maturing male parr are often among the faster growing members of the population but are later overtaken by females and immature males. Glebe and Saunders (1985) emphasized that conclusions concerning the relationship between growth and maturation await careful analysis of growth which has been adjusted to compensate for effects of maturation.

The observation that sexual maturation of juvenile male Atlantic salmon can commence and proceed under constant day length suggests that the initiation of testicular development may require a day-length threshold rather than increasing day length. If this is so, the LD 12:12 regime exceeds the threshold. Since our experiment did not include a shorter constant day length, e.g. LD 8:16, it is not clear whether a threshold day length is required for the initiation of sexual maturation in male parr. Saunders et al. (1982) and Saunders (1986) suggested that maturation of 0+ parr is dependent more on a developmental than a size threshold and that this threshold may be reached near the time of first feeding. It is possible, therefore, that sexual maturation among the males may have begun prior to the initiation of photoperiod regimes on May 24, i.e. before swim-up and first feeding.

Previous experiments studying the effects of photoperiod patterns of sexual maturation in salmonids have been started after the fish have lived for some time under natural photoperiods (Henderson 1963; Lundqvist 1980; Billard et al. 1981; Bromage et al. 1982; Scott et al. 1984; Billard 1985; Skarphedinsson et al. 1985; Duston and Bromage 1986). These experiments showed that it is possible to advance or delay sexual maturation. Extended or increasing day length most often stimulated early maturation in rainbow trout (*Salmo gairdneri*)

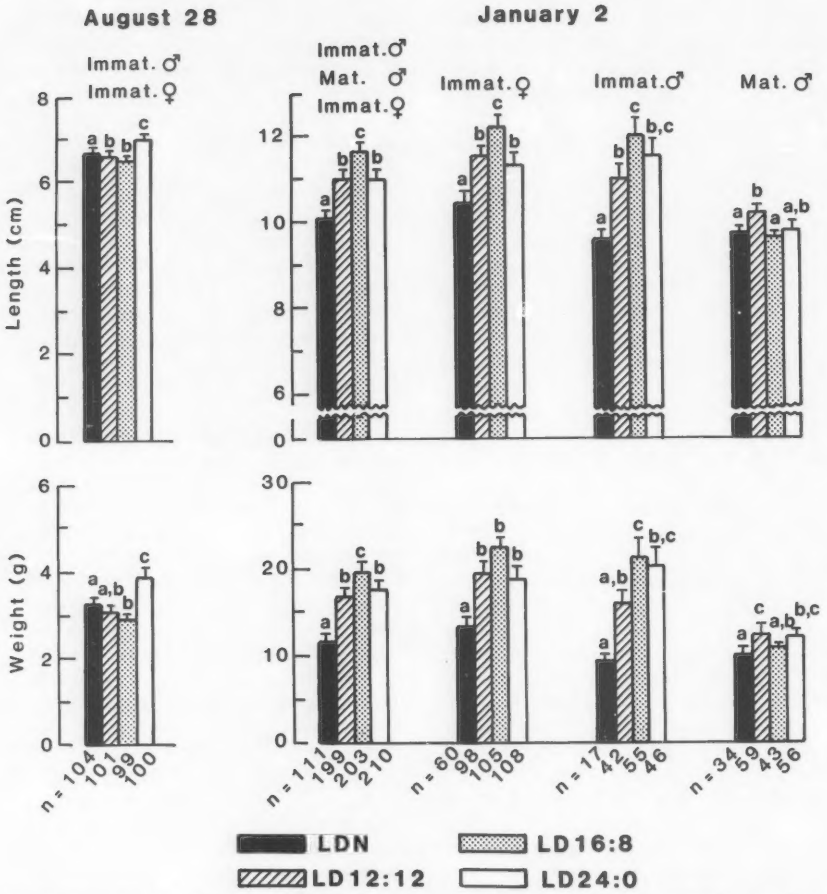

FIG. 1. Mean length and weight (+SE) of underyearling Atlantic salmon reared from first feeding (May 24) under three constant light regimes (LD 12:12, LD 16:8, and LD 24:0) and simulated natural photoperiod (LDN). Initial measurements June 20: length, 3.37 cm; weight, 0.43 g. Data for August 28 based on live fish; those for January 2 based on killed samples allowing breakdown according to sex and mature males. Note different ordinate scales for August and January values. Day-length treatment values sharing the same letter are not significantly different ($p > 0.05$).

(Skarphedinsson et al. 1985). However, these authors reported that a small proportion of males became sexually mature whatever the photoperiod. They suggested, further, that in rainbow trout there is a photosensitive phase occurring some time after 14 h from dawn, explaining why they observed mature males only under photoperiods exceeding 14 h. Our results are of interest in view of Henderson's (1963) observation that artificially shortened day length resulted in early ripeness of brook trout (*Salvelinus fontinalis*) in their second but not in their first reproductive cycle. Although gonadal development and spawning of salmonids can be accelerated by artificially reduced day length, sexual maturation appears to be initiated during the season of increasing day length (Scott and Sumpter 1983). That gonadal maturation cycles can be initiated and run under constant LD cycles and continuous light is in agreement with suggestions by Eriksson and Lundqvist (1982) and Clarke et al. (1985) that physiological processes such as smoltification and sexual maturation are rate controlled by increasing and decreasing day length, respectively, but that the initiation of these processes is controlled by endogenous rhythms. Further study should be aimed at more detailed observations of sexual maturity in relation to growth rate under various day-length regimes including those suspected of being below the threshold for initiation of maturation.

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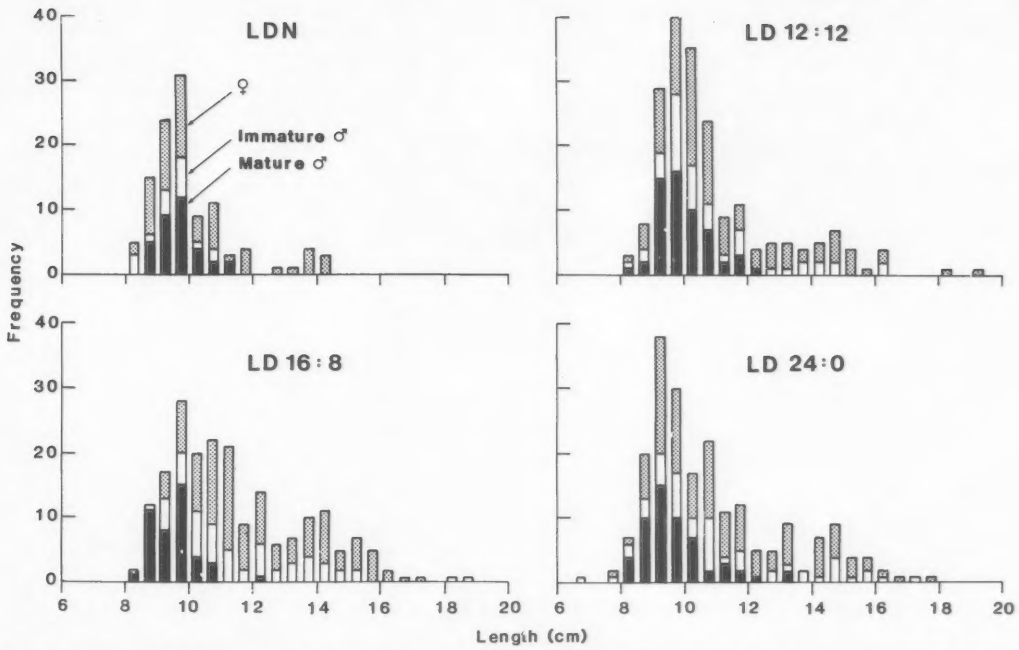

FIG. 2. Length–frequency distribution in early January of underyearling Atlantic salmon reared under three constant day length regimes and simulated natural photoperiod.

TABLE 1. Incidence of sexually mature male parr in early January among groups of underyearling Atlantic salmon reared under three constant photoperiods and simulated natural photoperiod.

Photoperiod regime	No. of females	No. of males	No. of mature males	Percent mature males
LDN	60	51	34	66.7[a]
LD 12:12	98	101	59	58.4
LD 16:8	105	98	43	43.9[a]
LD 24:0	108	102	56	54.9

[a]Significantly different ($p < 0.05$) from each other.

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Effects of Water Acidity on Swimbladder Function and Swimming in the Fathead Minnow, *Pimephales promelas*

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Jansen, W. A., and J. H. Gee. 1988. Effects of water acidity on swimbladder function and swimming in the fathead minnow, *Pimephales promelas*. Can. J. Fish. Aquat. Sci. 45: 65–77.

Swimbladder function, buoyancy-related behavior, and swimming performance were examined in fathead minnows (*Pimephales promelas*) following chronic (>4 d) exposure to acid-treated water (pH 5.3). When denied surface access in still water, treated fish, unlike controls (pH 7.7), failed to increase buoyancy and standard volume over "access to air" levels and had significantly higher proportions of swimbladder CO_2 and O_2. In current, treated fish reduced buoyancy over 48 h to a lesser extent than controls and were severely limited in their ability to increase internal pressure of swimbladder gases. pH significantly affected the maintenance of a minimum buoyancy over 32 d. Upon transfer from current to still water without access to air, the rate of buoyancy adjustment over 48 h was significantly slower in treated fish. With surface access, fish of both groups filled swimbladders within 6–12 h following removal from current; however, treated fish displayed significantly lower proportions of swimbladder CO_2 and O_2 at 12 and 24 h. Both groups of fish compensated hydrodynamically for insufficient static lift with higher frequencies of pectoral fin beats, treated fish having generally higher frequencies. Swimming performance was unaffected by water pH, but treated fish lost more weight than controls. We propose that impaired swimbladder function contributes to the elimination of fathead minnows from acidified environments.

Par suite d'une exposition chronique (>4 j) à de l'eau acide (pH 5,3), on a examiné la fonction de la vessie natatoire, le comportement lié à la flottabilité et le rendement natatoire chez le tête-de-boule (*Pimephales promelas*). En l'absence d'accès à la surface de l'eau calme et contrairement aux poissons témoins (pH 7,7), les poissons traités n'ont pu accroître leur flottabilité et leur volume normal par rapport au niveau « accès à l'air » et montraient des teneurs plus élevées en CO_2 et O_2 dans la vessie natatoire. En présence d'un courant, les poissons traités ont moins réduit leur flottabilité après 48 h que les poissons témoins; de plus, leur capacité d'augmenter la pression interne des gaz contenus dans la vessie natatoire était fortement limitée. Le pH a nettement touché le maintien d'une flottabilité minimum au cours d'une période de 32 j. Par suite du transfert des eaux courantes à des eaux tranquilles où l'accès à l'air était impossible, le taux d'ajustement de la flottabilité après 48 h était nettement plus faible chez les poissons traités. En présence d'un accès à la surface, les poissons des deux groupes ont rempli leur vessie natatoire de 6 à 12 h après leur retrait des eaux courantes; toutefois, on a observé des teneurs nettement plus faibles en CO_2 et O_2 dans la vessie natatoire des poissons traités après 12 et 24 h. On a noté des fréquences plus élevées de battement des nageoires pectorales, soit une compensation hydrodynamique pour le manque de portance statique, chez les deux groupes de poissons, et en particulier chez les poissons traités. Le rendement natatoire n'était pas touché par le pH de l'eau mais les poissons traités ont perdu plus de poids que les témoins. Selon les auteurs, la fonction modifiée de la vessie natatoire contribue à la disparition du tête-de-boule dans les environnements acides.

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Recent investigations on impacts of acid precipitation (acid rain) on aquatic ecosystems demonstrate that despite the occurrence of occasional fish kills (Jensen and Snekvik 1972; Leivestad and Muniz 1976; Grahn 1980), sublethal effects seem to be of greater ecological significance. Considerable research examines effects of such stresses on lake-dwelling organisms, but relatively little is directed towards fish in rivers and streams. Many fishes, however, spend part or all their lives in running water, where they must resist the downstream movement of current and hold position in order to survive and reproduce. Volume of discharge and water velocities are high during spring runoff, when input of acids from snowmelt is greatest, producing the lowest annual pH levels (Jeffries et al. 1979).

When exposed to increased water velocity, fish swim faster, holding position and generating hydrodynamic lift via the camber of their bodies and paired fins (Webb 1975; Magnuson 1978). As a result, total lift (hydrodynamic and static) may exceed that required to offset weight. To counter this, fish can swim at a negative angle of attack (Berezay and Gee 1978), reducing lift, but at the expense of additional drag and higher energetic costs (Alexander 1972). Alternatively, fish can reduce total lift by decreasing swimbladder volume (Saunders 1965; Neave et al. 1966; Gee et al. 1974) and enjoy lower energetic costs (Alexander 1972). When fish encounter lower velocity or still water, hydrodynamic forces are reduced, and fish must

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generate additional lift by increasing swimbladder volume or hydrodynamic forces (swimming) to maintain position in the water column. Shifting the primary source of lift rapidly by altering swimbladder volume is adaptive in terms of reducing drag because swimbladder lift is more efficient when swimming slowly in still water, and hydrodynamic lift is most efficient at fast swimming speeds when holding position in current (Gee 1983).

We examined the effects of sublethal acid stress on three ecologically significant components of hydrostatic buoyancy adjustment, related swimbladder parameters, behavior, and swimming ability in the fathead minnow (*Pimephales promelas*), a common cyprinid which is sensitive to reduced pH (Mount 1973). They can alter swimbladder volume extensively in response to changes in water velocity (Gee 1977) using both gas secretion and air gulping to enlarge the swimbladder and gas spitting and resorption to reduce its volume (Stewart and Gee 1981). Particular attention is directed at the (1) extent of buoyancy adjustment (the difference between minimum buoyancy in current and maximum buoyancy in still water) which ultimately affects the efficiency of locomotion and holding position, (2) rate of change in buoyancy which is important for the same reason, (3) ability to maintain minimum buoyancy over an extended time (weeks) which is critical for species living in rivers where spring runoff occurs over a long period, and (4) swimming performance in current. In this study, buoyancy is used to denote lift from the swimbladder. The components of buoyancy adjustment are affected by individual characteristics (size, age, etc.) and environmental factors (temperature, photoperiod, etc.; Gee 1983). Data pertaining to the effects of environmental pollutants, specifically water acidity, are not available. However, a number of studies have demonstrated severe physiological (e.g. haematological) disturbances for fish exposed to reduced pH (Wood and McDonald 1982). Considering the intimate link between mechanisms responsible for gas reabsorption and secretion in the swimbladder, and the chemical, as well as physical properties of fish blood (Steen 1970), the potential for impaired function of these processes is apparent.

Materials and Methods

Fathead minnows were collected from the Brokenhead River, Manitoba, in September 1982 and thereafter from City of Winnipeg water retention ponds. Fish were held in fiberglass tanks at 10–12°C under a 12 L : 12 D photoperiod and fed Tetramin flakes daily. Only fish with a coefficient of condition (K; Hile 1936) of 1 (range 0.94–1.02; $K = (W \cdot L^{-3})\ 100$, where W is wet weight in air (±0.01 g) and L is total length (±0.05 cm)) and total lengths between 5.05 and 7.10 cm were used in experiments.

Experimental Facilities

At least 10 d prior to testing, fish were transferred to 80-L holding aquaria and acclimated to a 14 L : 10 D photoperiod and to 19.5 ± 1.5°C by daily increments of 1°. Light phase (40-W bulbs) extended from 0730 to 2130. All experiments were conducted in either still water or stream tanks that simulated a running water environment. Still water aquaria (55 × 40 × 43 cm) contained only gently bubbling airstones. In stream tanks (85 × 45 × 57 cm), current was created using a design modified from Gee and Bartnik (1969) in which fish were held in current chambers (48 × 33 cm) with plastic screens at both ends and Plexiglas sides and bottom. Water depth varied from 2.5 to 3.5 cm, depending on velocity, which was determined by averaging 10 measurements from six different locations, each taken 2 cm from the bottom using an Ott current meter type Cl. Unless otherwise noted, water velocities were 34.3 $cm \cdot s^{-1}$ (range 31.1–38.0) for treated and 35.2 $cm \cdot s^{-1}$ (range 31.7–38.8) for control fish. In some still water experiments, access to the surface and to air bubbles was denied by using either 3-mm-mesh plastic screens (fish held in still water only) or by submersing smaller aquaria (55 × 25 × 28 cm), covered with perforated plastic lids, within still water tanks (fish held in still water transfer from current). Metal components did not come in contact with the test water.

Dechlorinated Winnipeg tap water was used for all experiments. Fish were held and tested at nominal pH values of 7.7 (control) or 5.3 (treated) and received at least 2 L $water \cdot g^{-1} \cdot d^{-1}$. A pH of 5.3 was chosen because it is sufficient to cause sublethal stress in fathead minnows but does not affect their survival (Mount 1973). For controls, two still water and one current aquaria received inflow directly from the tap. Water at pH of 5.3 was provided to three indentical aquaria as part of a circulation system, supplied from a 450-L storage tank via an 8-L mixing chamber. On initial filling, storage tank water was acidified (H_2SO_4) to pH 4.0, air-stripped of excess CO_2 for 2 d, and back-titrated to the test pH with NaOH. A pH combination electrode (Cole-Parmer K-5991-12) connected to an automatic pH controller (Chemcadet model 5652-00) continuously measured pH in the mixing chamber and, if required, activated peristaltic pumps supplying either H_2SO_4 or NaOH to the storage tank. In addition, pH in all aquaria was recorded twice daily and adjusted manually if required. Average pH (range) readings were 5.31 (5.22–5.46) and 7.68 (7.51–7.78) in still water aquaria and 5.33 (5.20–5.40) and 7.91 (7.70–8.24) in stream tanks.

The storage tank was filtered and continuously aerated to equilibrate water with atmospheric CO_2 and maintained at 19.5 ± 1°C by thermostat and heater. Depending on the duration of the experiment, water was exchanged from the circulation system after 16–47 d. The chemical characteristics of the water (Table 1) were determined from control and treated aquaria 10 d after the start of each set of experiments using standard analytical methods (Stainton et al. 1977).

Treated fish were initially exposed to increased acidity by supplying storage tank water to experimental aquaria at a rate of 100 $mL \cdot min^{-1}$, depressing pH from 7.7 to 5.3 within 2–3 d. Fish were then acclimated to pH 5.3 for at least 4 d and were not fed for 24 h prior to examination or for 12 h prior to transfer to stream tanks. Fish were sampled randomly and those with well-developed secondary sexual characteristics were discarded. In stream tanks an acclimation period of 12 h was allowed before initiating current. All experiments started at 0830, minimizing possible effects of diel activity rhythms. Except during swimming performance trials, water velocity was increased every 15 min for 1 h until the desired value was reached. Fish mortality was recorded daily in still water aquaria and was calculated as percent dead fish per week. Fish mortality in stream tanks (percentage stocked fish) was recorded three times daily.

Swimbladder and Behavioral Variables

Swimbladder variables determined were buoyancy, standard volume, internal pressure of gases, and gas composition. Buoyancy provides a measure of the proportion of body weight in

TABLE 1. Chemical characteristics of experimental water. Values are means (n = 5) with ranges given in parentheses; n = 1 for stream tanks. DIC = dissolved inorganic carbon; Cond. = conductivity.

Analysis	pH 7.7		pH 5.3	
	Still water	Stream tank	Still water	Stream tank
NH_4-N ($\mu g \cdot L^{-1}$)	37.5	30.0	1848	1240
	(30.0–50.0)	(—)	(760–2800)	(—)
Na^+ ($mg \cdot L^{-1}$)	1.94	2.07	10.17	2.31
	(1.80–2.18)	(—)	(2.37–38.40)	(—)
K^+ ($mg \cdot L^{-1}$)	1.37	1.42	2.13	1.62
	(1.30–1.46)	(—)	(1.46–2.90)	(—)
Ca^{2+} ($mg \cdot L^{-1}$)	20.5	18.7	26.8	25.0
	(13.6–22.9)	(—)	(15.1–36.8)	(—)
Mg^{2+} ($mg \cdot L^{-1}$)	6.57	8.16	7.96	6.14
	(5.19–9.28)	(—)	(6.31–10.9)	(—)
Cl^- ($mg \cdot L^{-1}$)	4.83	6.07	6.38	5.10
	(3.50–7.50)	(—)	(0.60–15.0)	(—)
SO_4^{2-} ($mg \cdot L^{-1}$)	5.35	3.60	101.8	83.5
	(3.50–7.20)	(—)	(13.9–175.0)	(—)
DIC ($\mu mol \cdot L^{-1}$)	1238	1380	36	50
	(690–1600)	(—)	(20–50)	(—)
Cond. (25°C) ($\mu S \cdot cm^{-1}$)	170	180	298	210
	(160–190)	(—)	(220–450)	(—)

water supported by the swimbladder, standard volume allows for a weight-independent comparison of swimbladder volume, and internal pressure reveals if fish regulate buoyancy by compression/expansion of swimbladder gas. To measure swimbladder variables, groups of three to five fish were captured at a time and anesthetized with a 0.03% solution of triacaine methanosulphate (MS 222). The anesthetic solution and water bath in which measurements were taken were similar in temperature (±2°) to experimental tanks.

Buoyancy was calculated similar to the procedure of Gee (1970) by dividing the swimbladder volume (±0.001 mL) by the weight of the gas-free fish in water (±1.0 mg), where 1.0 $mL \cdot g^{-1}$ is equivalent to neutral buoyancy. The difference between the weight of the fish in water with its swimbladder inflated and deflated was used to determine swimbladder volume. The volume of gas released from the swimbladder at atmospheric pressure (pascals) was measured by rupturing the swimbladder wall and capturing the gas under water with a watch glass suspended from an under-the-balance hook. The difference in weight (grams) of the watch glass before and after the gas was released was equivalent to the gas volume (millilitres). The released volume of gas, corrected to 101.3 kPa (standard pressure), was divided by the weight of the gas-free fish in water to provide a measure of the weight-specific swimbladder gas volume (standard volume). Internal pressure was calculated by dividing the volume of the free swimbladder gas at atmospheric pressure by the swimbladder volume. Swimbladder gas composition was measured following the procedure of Stewart and Gee (1981) using a Carle model 8700 gas chromatograph.

Since it was not possible to measure all swimbladder parameters on the same fish, experiments requiring gas composition analysis were repeated, the first providing results on buoyancy, standard volume, and internal pressure and the second providing data on gas composition, buoyancy, and standard volume. As a result, in some experiments, two data sets were available on buoyancy and standard volume. These will be described and discussed separately and will be referred to as buoyancy and gas composition experiment.

Fish in still water at near-neutral buoyancy and highly negatively buoyant fish, transferred from current into still water, were observed for pectoral fin beat frequency (PFBF) in treated and normal water to determine the ability to generate hydrodynamic lift from paired fins. Except when gulping air, most of the fish in still water hovered in midwater while adopting a horizontal or slightly inclined position with the caudal peduncle above the head. Fish then beat their pectoral fins and, to a lesser extent, used undulating movements of the lower lobe of the caudal fin to maintain position in the water column. The pectoral fins beat synchronously with minor apparent differences in amplitude and phase difference between individual fin rays. This motion was termed oscilliatory by Blake (1983). The effective stroke of the beat was downwards. PFBF was measured while fish hovered by counting from behind a blind, each paired stroke (down and up) over a time period varying from 5 to 60 s. Variation in beat amplitude was not taken into account.

Experimental Procedures

To examine the extent of adjustment in buoyancy, standard volume, concomitant internal pressure, gas composition, and PFBF, groups of 10–18 control and treated fish were held in still water with and without access to air for 10–15 d or in current for 48 h after acclimation to specific pH conditions in still water (Table 2). Access to air was denied to assess the contribution of air gulping and gas secretion on swimbladder inflation. PFBF was measured in still water at least three times for each fish at 1900–2000 before the morning of sampling.

Rates of adjustment of swimbladder parameters and PFBF were determined via time-series experiments, in which groups of 10–17 fish were sampled and measured at 0, 1, 3, 6, 12, 24, and 48 h after the initiation of current (decrease in buoyancy) or the transfer from current to still water with or without access to air (increase in buoyancy; Table 2). Except for the buoyancy experiment on nonaccess fish (Jansen 1986), observations of PFBF were made continuously for the first hour in still water after transfer from current and then for 30-min intervals commencing at 2, 5, 11, 23, and 47 h, if applicable. As approximately 40% of the nonaccess fish in the buoyancy experiment died after 48 h in current, a decreased water velocity of 25.8

TABLE 2. Summary of experimental design. Arrows indicate transfer between holding conditions. STV = standard volume; IP = internal pressure; SGC = swimbladder gas composition; SWT = swimming time.

Experiment and holding conditions	Access to air	Time of observation	Measured variables
1. Extent of buoyancy adjustment			
(a) Still water	Yes	10–15 d	Buoyancy, STV,
	No	48 h	Buoyancy, STV, IP, SGC
(b) Current	Yes	48 h	Buoyancy, STV, IP, SGC
2. Rate of buoyancy adjustment			
(a) Still water → current (10–15 d)	Yes	1,3,6,12,24,48 h	Buoyancy, STV, IP
(b) Current → still water (48 h)	Yes	1,3,6,12,24 h	Buoyancy, STV,
	No	1,3,6,12,24,48 h	IP,SGC,PFBF
3. Maintenance of a reduced buoyancy current	Yes	1,2,3,6,12,20,32 d	Buoyancy, STV, IP
4. Swimming performance Current of stepwise increasing velocity	Yes	10 h	Buoyancy, IP, SWT, weight, K

± 2.9 (control) and 26.4 ± 3.2 cm·s^{-1} (treated) was used for access fish and all fish of the gas composition experiment. Swimbladder parameters were not significantly affected by this change in water velocity (Jansen 1986).

To determine the ability to maintain a minimum buoyancy and standard volume, or a particular internal pressure over an extended period of time, fish were examined in groups of 11–17 after 1, 2, 3, 6, 12, 20, and 32 d in current (Table 2). Water velocity was 25.8 ± 2.9 cm·s^{-1} for control and 26.4 ± 3.2 cm·s^{-1} for treated fish. While in current chambers, fish were fed three times daily, the last feeding 48 h before examination.

Rapid adjustment and maintenance of a specific swimbladder lift reduces energy expenditure in locomotion (Gee 1987). Therefore, differences in the ability to modulate buoyancy between control and treated fish should be reflected in swimming performance and/or condition after exposure to current. To measure these, four groups (n = 10) each of treated and control fish were sampled from the still water aquaria, anesthetized in MS 222, and their coefficient of condition (K) determined. Sketches made of individual fishes facilitated later identification. Fish of similar K were placed into the current chamber which had a low water flow of 2 cm·s^{-1} and were allowed to acclimate for 12–14 h. At 0815 on the day of testing, a gentle current (10 cm·s^{-1}) was initiated. Water velocity was increased to 15.2 ± 2.5 cm·s^{-1} at 0830 and from then at 2-h intervals to 25.3 ± 3.5, 35.2 ± 3.5, 43.7 ± 4.6, and 57.5 ± 5.5 cm·s^{-1}. Fish were observed almost continuously and individuals resting against the back screen for 60 s without reacting to a gentle prodding were removed. Because fish could not tolerate the highest water velocity for the full 2 h, an experiment was terminated after the last fish was removed. Swimming time for each fish was recorded from 0830 to the time of exhaustion to the nearest 10 min together with its weight, buoyancy, and internal pressure (Table 2). A new condition factor was calculated for each fish at the end of the experiment.

Data Analysis

Statistical analyses, except tests of variance equality, were run with the Statistical Analysis System (SAS 1982). Differences between pH treatments and between time periods were tested by analysis of variance (ANOVA), Student's t-test, or least square regression analysis. If ANOVA revealed that group means were significantly ($p \leq 0.01$) different, orthogonal comparisons using F-tests as tests of significance were applied to distinguish differences between means for control and treated fish for each time period. Duncan's new multiple range test was used to test differences between time intervals within pH treatments. A probability level of ≤0.05 was considered significant. To obtain information on rate of change, parameters were regressed against time with the independent variable being transformed to $\sqrt{X}$ and $\log(X+1)$; adding 1 to values accounted for time zero. Untransformed and transformed time variables and their second and third degree powers were used to find the best-fit model. When applicable, slopes of regression models were tested for parallelism. Homogeneity of group variances was tested applying Hartley's F_{max}-test (Sokal and Rohlf 1981). If variances were not homogeneous, data were log- or arcsin-transformed, which stabilized the variances. For clarity of presentation, arithmetic means of the untransformed data with 95% confidence intervals have been used in all figures and tables.

Results and Discussion

Mortality

Of 1252 fish held in the still water aquaria at pH 5.3 (n = 618) and pH 7.7 (n = 634), 38 treated and 41 control fish died before being sampled or transferred to the stream tank. Weekly mortality rates varied between 1 and 5% for both groups and did not vary with time (n = 4–42 d). Mortality in current increased with water velocity and over time, but was independent of water acidity (Jansen 1986). This pattern also

indicates that the high ammonia levels in the treated recirculated water had no significant effect. At pH 5.3, only a very small amount of ammonia is present as toxic undissociated NH_4OH (Trussell 1972). Survival of fathead minnows corresponded to results of a laboratory study by Mount (1973) at pHs of 4.5–6.5. In contrast, Zischke et al. (1983) reported 50% lower survivorship of acid-exposed (pH 5.0) fathead minnows compared with controls.

Variables in Still Water and Current

In still water with surface access, most measured variables were similar in control and treated fish (Table 3). In agreement with results by Gee et al. (1974), Gee (1977), Stewart and Gee (1981), and Chiasson and Gee (1983), buoyancy was slightly negative in both groups, indicating that fathead minnows must generate some hydrodynamic lift to remain pelagic. Control fish, however, maintained significantly higher internal pressures than treated fish, at levels just above atmospheric. Proportions of CO_2 were similar, but treated fish had significantly ($p < 0.01$) higher levels of O_2 and lower levels of N_2. PFBF was not significantly ($p = 0.10$) affected by pH, both groups of fish beating their pectoral fins about 1 beat·s^{-1}. In still water without surface access, control fish were almost neutrally buoyant whereas treated fish had significantly lower standard volume ($p < 0.001$) and buoyancy ($p < 0.0001$) (Table 3). Internal pressure was similar in both groups. Proportions of CO_2 and O_2 were elevated ($p < 0.0001$), and percent N_2 was lower ($p < 0.0001$) in treated fish. PFBF of treated fish was higher ($p < 0.01$). Without access to air, control fish increased buoyancy ($p < 0.01$), standard volume ($p < 0.05$), and percent O_2 ($p < 0.01$) compared with control fish with access whereas percent N_2 decreased ($p < 0.01$) (Table 3). In treated fish, buoyancy remained unchanged, but the proportions of O_2 and CO_2 were elevated ($p < 0.0001$) and the percent N_2 correspondingly reduced. PFBF of both treated ($p < 0.005$) and control ($p < 0.05$) fish were higher than for similar fish with access to air (Table 3).

Swimbladder gas composition of control fish with access to air (Table 3) was typical for fathead minnows (Stewart and Gee 1981; Chiasson and Gee 1983) and conformed to the general cyprinid pattern of relatively high CO_2 (2.0–5.8) and low O_2 (3.4–12.3) percentages (Hüfner 1892; Hall 1924; Evans and Damant 1929; Jacobs 1934; Krohn and Piiper 1962). In agreement with previous reports on a high impermeability of the swimbladder wall (Moreau 1876; Evans and Damant 1929; Kutchai and Steen 1971; Denton et al. 1972; Ross 1979a; Wittenberg et al. 1980), the gas composition of control fish suggests that under normal conditions (i.e. still water with surface access), diffusional gas losses are minor. Otherwise, losses would require compensation by either air gulping or gas secretion. Direct observations showed air gulping to be negligable (Jansen 1986). Like many other species (Akita 1936; Scholander 1956; Steen 1963b; McNabb and Mecham 1971), fathead minnows secrete swimbladder gas consisting mainly of O_2 and, to a lesser extent, CO_2 and N_2 (see below). Any substantial gas secretion will therefore lead to elevated swimbladder O_2 and CO_2 percentages compared with the composition of air. Such concentrations were not found in still water control fish. It seems that unstressed fathead minnows can maintain a constant swimbladder volume at very low metabolic costs. Such costs have been estimated by Alexander (1972). Similarly, swimbladder gas composition of treated fish with access to air suggests that buoyancy and standard volume could only be maintained at the expense of increased rates of gas secretion, supplemented by elevated numbers of air gulps (Jansen 1986). Denial of surface access apparently increased these regulatory problems in treated fish and also affected controls, resulting in elevated gas secretion. In controls, this volume of gas seemed to be relatively small and served to increase standard volume and, therefore, buoyancy. The apparent lack of change in these two parameters in treated fish is surprising, because their very high CO_2 and O_2 percentages suggest substantial gas secretion over an extended period of time. One possible explanation is extensive gas loss from the swimbladder, equalling the rate of gas secretion. Gas reabsorption occurs passively by diffusion into the blood (Moreau 1876) and is regulated by the degree of closure of a relatively impermeable tissue layer and by constriction or dilation of the underlying capillaries (von Ledebur 1937). The degree of vasoconstriction and muscular contraction is controlled by autonomic reflexes and hormones (Fänge 1973; Ross 1978). Whether acid exposure affects these control mechanisms or the gas permeability of the swimbladder wall is not known.

TABLE 3. Effect of water pH on buoyancy, standard volume, internal pressure, swimbladder gas composition, and PFBF for fathead minnows held in still water for 10—15 d with and without access to air. Values represent means ± 95% confidence interval (n = number of fish tested, except for PFBF where n = number of repeated observations). *Significantly (t-test, $p < 0.05$) different from access to air at the same pH. **Significantly (t-test, $p < 0.05$) different from pH 7.7.

	Access to air		No access to air	
	pH 7.7	pH 5.3	pH 7.7	pH 5.3
Buoyancy (mL·g^{-1})	0.886±0.053 (n=12)	0.862±0.046 (n=13)	0.976±0.013* (n=12)	0.870±0.035** (n=12)
Standard volume (mL·g^{-1})	0.874±0.057 (n=12)	0.865±0.048 (n=13)	0.947±0.016* (n=12)	0.860±0.035* (n=12)
Internal pressure (atm)	1.012±0.006 (n=12)	1.001±0.006** (n=13)	1.008±0.010 (n=12)	1.001±0.011 (n=12)
CO_2 (%)	2.86±0.32 (n=17)	2.52±0.40 (n=16)	3.10±0.54 (n=14)	4.88±0.48*,** (n=13)
O_2 (%)	13.81±3.14 (n=17)	23.84±4.13** (n=16)	20.57±4.34* (n=14)	45.51±5.36*,** (n=13)
N_2 (%)	83.33±3.16 (n=17)	73.63±4.35** (n=16)	76.32±4.73* (n=14)	49.60±5.60*,** (n=13)
PFBF (beats·min^{-1})	55.88±5.45 (n=68)	66.34±7.56 (n=50)	68.84±7.61* (n=32)	91.74±15.34*,** (n=27)

TABLE 4. Effect of water pH on buoyancy, standard volume, internal pressure, and swimbladder gas composition for fathead minnows after 48 h in current. Values represent means ± 95% confidence interval (n = number of fish tested). *Significantly (t-test, $p < 0.05$) different from pH 7.7.

	pH 7.7	pH 5.3
Buoyancy ($mL \cdot g^{-1}$)	0.374 ± 0.049 (n = 13)	0.469 ± 0.067* (n = 12)
Standard volume ($mL \cdot g^{-1}$)	0.389 ± 0.051 (n = 13)	0.468 ± 0.072 (n = 12)
Internal pressure (atm)	1.057 ± 0.015 (n = 13)	1.014 ± 0.016* (n = 12)
CO_2 (%)	1.19 ± 0.20 (n = 11)	1.24 ± 0.17 (n = 13)
O_2 (%)	8.02 ± 2.27 (n = 11)	9.53 ± 1.75 (n = 11)
N_2 (%)	90.81 ± 2.25 (n = 11)	89.23 ± 1.67 (n = 11)

Wood and McDonald (1982) noted the paucity of information available on endocrinological effects and the absence of neurophysiological studies. Whatever the reason, treated fish in still water without access to air can maintain buoyancy only at the expense of extensive gas secretion and mechanical costs of increasing hydrodynamic lift via elevated PFBF.

Over 48 h in current, fish reduced buoyancy to 54.4 (treated) and 42.2% (control) of still water values (Tables 3 and 4), results in agreement with those of Gee (1977) and Stewart and Gee (1981) for neutral pH conditions. Control fish were significantly less buoyant than treated fish (Table 4). This difference mainly resulted from a higher ($p < 0.0001$) internal pressure among control fish, as standard volumes for both groups were similar. Gas compositions of control and treated fish were similar (Table 4). O_2 and CO_2 percentages for both groups were less ($p < 0.01$) than in still water, while those of N_2 were increased ($p = 0.001$) (Tables 3 and 4). Similar to results by Gee (1977), control fish increased ($p < 0.0001$) internal pressure between still water and current. Treated fish were unable to do so (Tables 3 and 4). The extent of adjustment, i.e. the ability to lower buoyancy in current, was significantly greater for control fish (0.512 $mL \cdot g^{-1}$) than for treated fish (0.393 $mL \cdot g^{-1}$) (Tables 3 and 4). The extent of adjustment in standard volume was not significant ($p = 0.09$). These results support the notion that under normal conditions, two mechanisms operate in buoyancy reduction of fathead minnows, reabsorption of gases (mainly O_2 and CO_2) and increase in internal pressure (Gee 1977). In treated fish, internal pressure did not contribute to buoyancy adjustment, thus reducing its extent.

Changes in Variables during Buoyancy Adjustment

In current for 48 h after acclimation to still water, treated and control fish reduced buoyancy and standard volume linearly with the logarithm of time (Table 5). Slope comparisons of regression equations indicated that control fish reduced buoyancy significantly faster than treated fish (Table 5). At 6 h or more, control fish had significantly lower buoyancies than treated fish (Fig. 1A). Similar results were found for standard volume, although differences between groups beyond 6 h were not significant at 48 h ($p = 0.06$; Fig. 1B). Internal pressure fluctuated considerably over time in current and followed similar patterns in control and treated fish (Fig. 1C).

In still water with access to air after acclimation to current, control and treated fish reached maximum buoyancy and standard volume after 6–12 h; however, treated fish were slightly less buoyant at 12 and 24 h (Fig. 1D and 1E). Water pH did not significantly affect these two swimbladder parameters over 1–24 h, but the interaction of time and pH was significant. With surface access, treated and control fish decreased internal pressure over time at similar rates (Table 5) to less than atmospheric values (Fig. 1F). pH affected percentage changes in CO_2 ($p < 0.05$), O_2 ($p < 0.01$), and N_2 ($p < 0.001$) over time; proportions of CO_2 and O_2 at 12 and 24 h were significantly lower and those of N_2 significantly higher in treated fish (Fig. 2). Maximum CO_2 and O_2 percentages were reached after 3 and 12 h, respectively.

On exposure to still water without access to air after acclimation to current, swimbladder O_2 and CO_2 percentges increased sharply, and faster than in fish with access (Fig. 2). Gas composition was not significantly affected by pH. Compared with values at 48 h in current, maximum O_2 and CO_2 concentrations, reached after 24 h, were increased approximately fivefold and threefold, respectively. Internal pressure fluctuated considerably over time in both group of fish, reaching similar minimum values after 48 h (Fig. 1J). Control and treated fish more than doubled buoyancy and standard volume over 48 h to return to long-term still water levels (Fig. 1G and 1H). For fish of both groups, buoyancy and standard volume increased linearly with the logarithm of time; however, the rates of increase were significantly slower in treated fish (Table 5). A similar result was obtained from the gas composition experiment (Jansen 1986). These rate differences suggest that the mechanisms of gas secretion are quantitatively affected by acid exposure. If changes in standard volume are taken as direct measure of swimbladder gas secretion, ignoring possible diffusional losses, controls secreted 22% more gas than treated fish. The latter may be able to compensate for their inferior rate of gas secretion by relying more extensively on air gulping to increase standard volume and, therefore, buoyancy. Several sources of evidence support this argument. Similar to nonaccess fish, the increase in standard volume of access control fish over 24 h was 33% larger than that of treated fish, and was accomplished while gulping air as often (buoyancy experiment) or less than (gas composition experiment) treated fish (Jansen 1986). Finally, proportions of swimbladder CO_2 and O_2 were higher in access control fish. Air brought into the swimbladder by gulping will mix with freshly secreted gas, proportions of CO_2 and O_2 being elevated as the contribution of gas secretion increases. By a similar argument, however, the swimbladder gas composition of fish without surface access cannot be interpreted in favor of differences in gas secretion rates between treated and control fish. The almost identical and, compared with atmospheric, very high CO_2 and O_2 proportions indicate that the mechanisms of gas secretion are not qualitatively affected by acid exposure and that the composition of freshly secreted gas is similar to that of other species (see earlier). Further work on gas secretion rates and gas compositon is required to more precisely define the qualitative and quantitative acid effects on the swimbladder.

During the first hour after transfer from current to still water, fish beat their pectoral fins at a rate of approximately 270 $beats \cdot min^{-1}$ (Table 6), independent of water pH or surface access. The frequency then declined and reached minimum values during the last observation period. For all data sets (access, pH combinations), the decline in PFBF was best described by

TABLE 5. Regression equations describing rates of change in buoyancy, standard volume, and internal pressure for fathead minnows on exposure to water current (after acclimation to still water) and on exposure to still water with and without surface access (after acclimation to current). *Significant (ANCOVAR, $p<0.05$) difference in slope between control and treated fish (x = log time in hours).

	pH 7.7	pH 5.3
	Current	
Buoyancy	$y = 0.816 - 0.299x$ ($r^2 = 0.74$)	$y = 0.830 - 0.240x$ ($r^2 = 0.68$)*
Standard volume	$y = 0.789 - 0.278x$ ($r^2 = 0.70$)	$y = 0.810 - 0.228x$ ($r^2 = 0.63$)
Internal pressure	n.s.	n.s.
	Still water with access	
Buoyancy	n.s.	n.s.
Standard volume	n.s.	n.s.
Internal pressure	$y = 1.049 - 0.050x$ ($r^2 = 0.53$)	$y = 1.026 - 0.035x$ ($r^2 = 0.22$)
	Still water without access	
Buoyancy	$y = 0.370 + 0.341x$ ($r^2 = 0.81$)	$y = 0.443 - 0.261x$ ($r^2 = 0.64$)*
Standard volume	$y = 0.370 + 0.325x$ ($r^2 = 0.80$)	$y = 0.437 - 0.249x$ ($r^2 = 0.61$)*
Internal pressure	n.s.	n.s.

curvilinear models, which accounted for between 64 and 82% of the observed variability ($p < 0.0001$). For fish without access to air, pH did not significantly influence mean PFBF; however, at 6 and 12 h the frequency of treated fish was significantly higher than that of controls (Table 6). For fish with access to air, buoyancy and gas composition experiments showed different results in terms of PFBF response. For the buoyancy experiment the effect of pH on PFBF was significant; commencing at 12 h, treated fish showed significantly higher frequencies (Table 6). No such effect was apparent for fish in the gas composition experiment. When PFBF was regressed on buoyancy, mean PFBF accounted for 71% of the variance in mean buoyancy, indicating that PFBF is a useful predictor of buoyancy. This relationship agrees with results by Jones (1952) and McCutcheon (1958) and suggests that static and hydrodynamic lift are directly complementary in providing neutral total lift. Therefore, cases in which treated fish displayed higher beat frequencies than controls of similar or lower buoyancy are difficult to explain. Perhaps acid exposure affects the ability to generate hydrodynamic lift per fin beat. Alternative explanations are inaccuracies in counting beat frequencies above 240·min^{-1} and/or the time delay between the measurements of beat frequencies and buoyancy. To evaluate the ecological significance of these results, it must be asked if, and to what extent, these laboratory observations reflect the behavior of fathead minnows in their natural habitat (Lindsey 1978). Fathead minnows feed on detritus, insect larvae, and small crustaceans (Held and Peterka 1974; Tallman et al. 1984). These food objects are immobile or slow moving and feeding involves slow swimming with intermittent hovering. Forward locomotion will not provide much lift under these conditions and extensive use of the pectoral fins seems a likely strategy for generating hydrodynamic lift.

Ability to Maintain a Minimum Buoyancy in Current Over Time

When exposed to water current over an extended period of time, both treated ($p < 0.0001$) and control ($p < 0.01$) fish significantly increased buoyancy and standard volume from day 2 (minimum values) to day 32 (Fig. 3). pH significantly ($p < 0.001$) affected buoyancy over time, while for standard volume the effect of pH was not quite significant ($p = 0.07$). In control fish, only buoyancy at day 32 was significantly higher than that at day 2 (Fig. 3). Both buoyancy and standard volume were significantly higher in treated fish than in controls at day 20 and 32 (Fig. 3). Internal pressure was significantly affected by pH, time, and their interaction. Internal pressure of treated fish was consistantly lower ($p < 0.001$) between day 2 and 20 than that of controls. Control fish increased internal pressure over this time period ($r^2 = 0.17$, $p < 0.01$). In treated fish, internal pressure remained at similar values from day 2 to 32. Internal pressure of control fish declined dramatically between day 20 and 32, attaining values similar to treated fish (Fig. 3). Stewart and Gee (1981) did not observe an increase in buoyancy in fathead minnows swimming at 20 or 30 cm·s^{-1} for up to 42 d. However, these experiments provided sheltered areas of reduced current (J. H. Gee, unpubl. obs.), thus reducing stress from fast swimming. Present mortality rates indicate the considerable stress imposed on fish by water velocity alone. Stress of increasing temperature and/or water velocity has been shown to adversely affect control of buoyancy (Gee 1977; Berezay and Gee 1978; Stewart and Gee 1981) and internal pressure (Stewart and Gee 1981). If, in control fish, this effect is critically manifested between day 20 and 32, the additional acid stress may influence both magnitude and timing of impairment in buoyancy control. This control is likely affected both by the loss of internal pressures greater than ambient and, to a lesser degree, by an increase in standard volume. Excess internal swimbladder pressure in the order of 20–130 mm Hg (Evans and Damant 1929; Franz 1937; McCutcheon 1958; Alexander 1959; Gee et al. 1974; Gee and Gee 1976) and with decreasing buoyancy increasing internal pressure (Gee 1970; Gee et al. 1974; Machniak and Gee 1975) has been reported for a number of species. McCutcheon (1958, 1966) suggested that muscular compression of the swimbladder wall changes internal pressure and thereby provides for precise swimbladder control. Mean internal pressures in excess of 26 mm Hg above atmospheric were never found in treated fish. It seems likely that acid-exposed fathead minnows are severely restricted in the use of this mechanism of precise buoyancy adjustment. If, and to what extent, the operation of the Weberian apparatus, which relies on a certain tension of the swimbladder wall (Evans and Damant

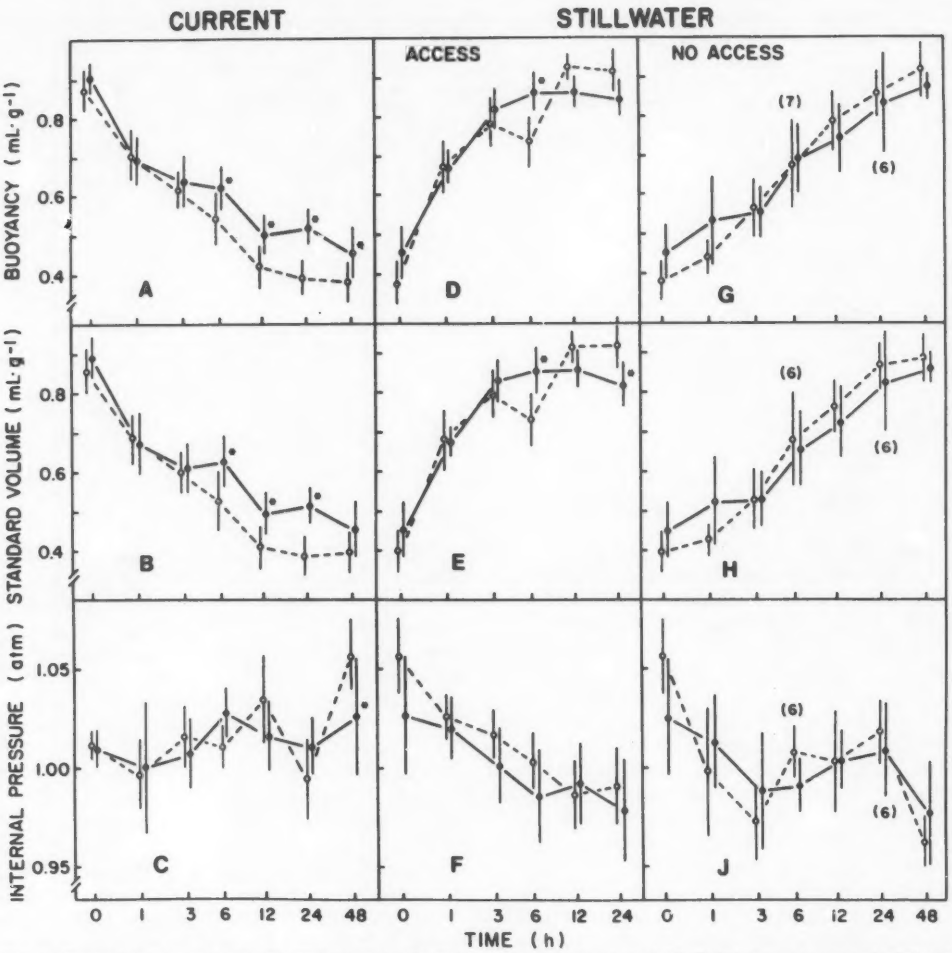

FIG. 1. Temporal changes in (A, D, G) buoyancy, (B, E, H) standard volume, and (C, F, J) internal pressure in fathead minnows exposed to pH 5.3 (solid symbols) and pH 7.7 (open symbols) in current (after acclimation to still water) and in still water (after acclimation to current). Points represent means ±95% confidence interval of 8–14 fish (except where indicated). Means significantly ($p < 0.05$) different from control are indicated with an asterisk. Time is plotted on a log scale and actual values are shown.

1929; Alexander 1959), is affected in acid-exposed cyprynids remains unknown.

Swimming Performance

Fish of all four replicates within each pH treatment responded similarly (ANOVA, $p > 0.1$) and data for all fish were pooled for analysis. When subjected to a stepwise increase in water velocity, mean swimming times of treated and control fish were similar (Table 7); all fish of both groups resisted water velocities of 15 and 25 cm·s^{-1}, three to four fish tired at 35 and 44 cm·s^{-1}, respectively, and none of the fish swam longer than 60 min in 57 cm·s^{-1} current. However, it must be recognized that the present measure of swimming time suffered limitations. At high current speeds, it became increasingly difficult to maintain an acceptable velocity profile and the magnitude of velocity increments was limited by the design of the stream tank. Obviously, a smaller step increase to the highest velocity would have resulted in more precise separation of swimming time for individual fishes. Furthermore, at 57.5 cm·s^{-1}, fish swept against the downstream screen blocked water flow and this resulted in a greatly increased velocity gradient. Considering these limitations, the similar swimming times of treated and control fish and the lack of relationship between buoyancy and swimming time do not preclude the possibility that acid-exposed fish fatigue more easily at a given water velocity or have lower prolonged swimming speeds. Both buoyancy (Berezay and Gee 1978) and pH (Graham and Wood 1981) have previously been shown to affect swimming performance. Although pH levels in the latter study were sufficiently low (see below) to attribute the reduced swimming performance of rainbow trout (*Salmo gairdneri*) directly to respiratory disturbances (Graham and Wood 1981), elevated energetic costs due to suboptimal buoyancy may have also contributed.

The approximately 7 h of forced swimming resulted in an average loss of 9.2 and 5.5% of body weight in treated and control fish, respectively. This difference was manifested in a reduced ($p < 0.001$) K of treated fish after the termination of

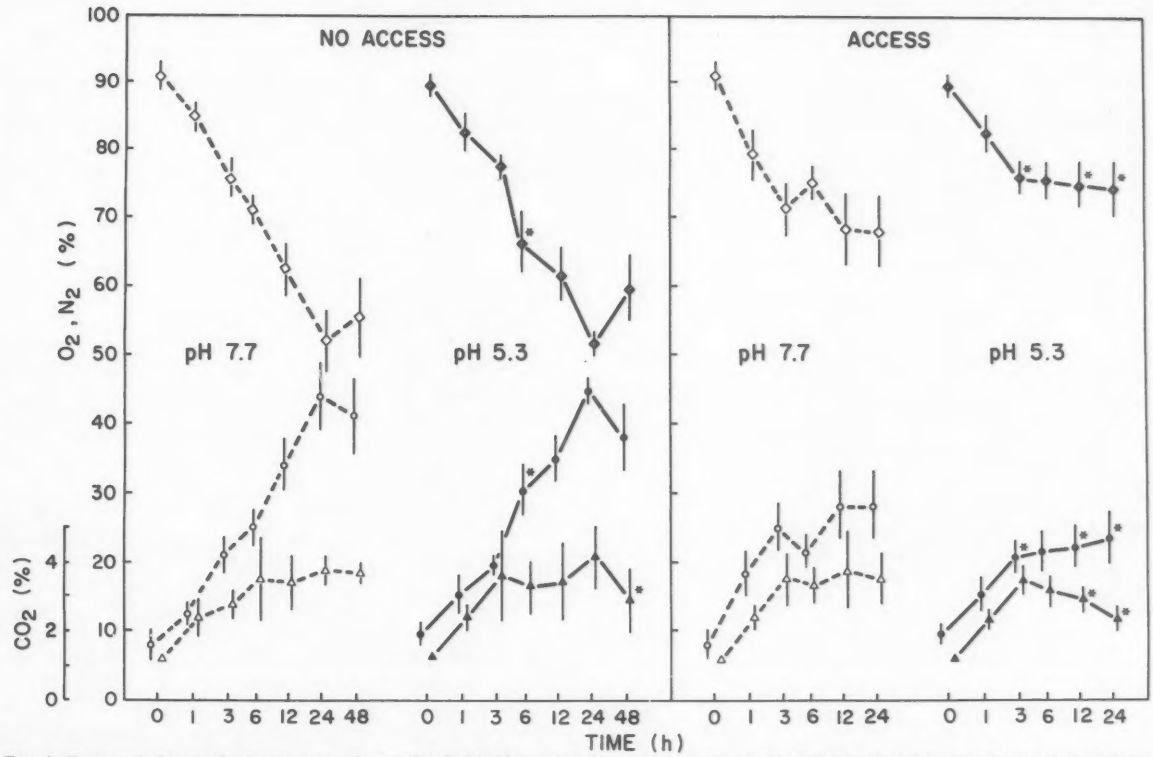

FIG. 2. Temporal changes in the percent volume of swimbladder gases of control (open symbols) and treated (solid symbols) fathead minnows during the return to neutral buoyancy in still water. Points represent means ±95% confidence interval of 10–14 fish for CO_2 (triangles), O_2 (circles), and N_2 (diamonds). Confidence intervals <0.2 are not shown. Means significantly ($p < 0.05$) different from control are indicated with an asterisk. Time is plotted on a log scale and actual values are shown.

TABLE 6. Temporal changes in mean PFBF (beats·min^{-1}) of fathead minnows during the return to neutral buoyancy in still water (after acclimation to current). Values represent means ± 95% confidence interval of 16–163 observations. A total of 2397 observations on 349 fish are included in the analysis. *Significantly ($p < 0.05$) different from pH 7.7.

Time interval (h)	Buoyancy experiment		Gas composition experiment			
	Access		Access		No access	
	pH 7.7	pH 5.3	pH 7.7	pH 5.3	pH 7.7	pH 5.3
0 –1	270.2±4.4	270.6±4.1	277.7±4.2	269.3±4.5	272.5±3.4	273.0±3.0
2.0–2.5	194.3±8.0	206.6±5.0*	210.6±15.0	199.5±12.1	259.9±4.4	265.5±4.8
5.0–5.5	174.8±9.4	151.6±10.6*	81.6±13.7	117.8±10.9*	237.7±7.7	256.0±4.3*
11.0–11.5	85.9±10.2	132.0±12.1*	70.9±15.7	67.8±7.5	208.8±12.2	219.6±10.5*
23.0–23.5	63.8±12.7	114.6±12.7*	63.4±20.7	65.8±12.4	112.0±17.9	116.8±20.7
47.0–47.5	—	—	—	—	76.7±9.9	80.7±17.7

the experiment (Table 7). It can be assumed that energy expenditure during locomotion is linearly related to swimming effort. In the present study, the amount of energy expended during exercise was probably less affected by the experimental conditions than the measure of swimming time. Changes in condition provide some indirect information on energy expenditure. Prior to the experiment, fish were not fed for 24–26 h. No specific data are available for fathead minnows, but according to Beamish (1978) this time period is sufficiently long to assure postabsorptive state. Consequently, energy requirements must have been met by metabolizing stored reserves. The composition of the weight loss is not known. Sockeye salmon (*Oncorhynchus nerka*) decreased lipid, protein, and caloric content per gram wet weight during sustained swimming (Brett 1973). It seems reasonable, therefore, to attribute the weight loss in fathead minnows to a reduction in caloric content of the fish. However, using O_2 consumption rates of fathead minnows during active metabolism (MacLeod and Smith 1966), energy equivalents of O_2 uptake, and energy derived from metabolized body stores (Brett and Groves 1979), it can be calculated that the theoretical weight loss in form of fat and protein is in the order of 0.1 g and cannot fully account for actual weight differences. Elevated O_2 consumption rates over steady-state conditions or partial metabolism of body fuels (Brett 1973) may be responsible for these differences. Possibly, acid exposure also has differential effects on body water loss. Considering the

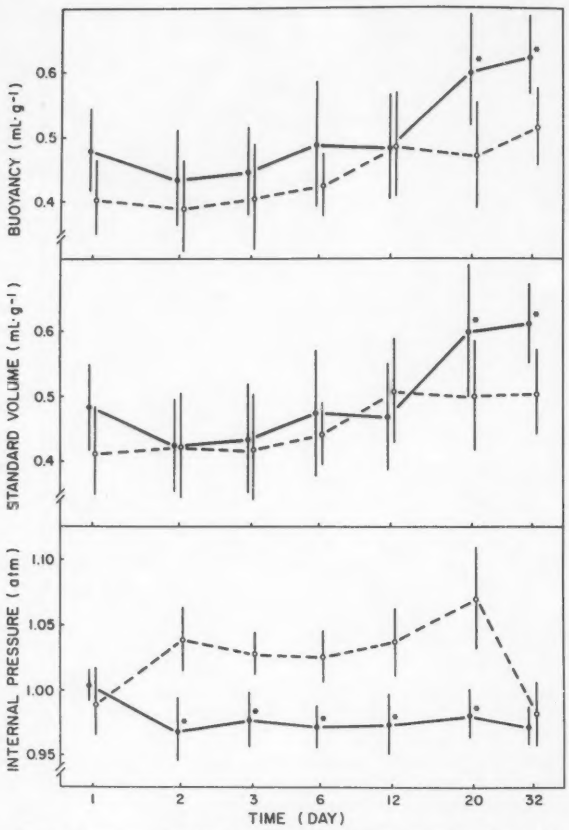

FIG. 3. Buoyancy, standard volume, and internal pressure after 1–32 d in current at pH 5.3 (solid symbols) or pH 7.7 (open symbols). Points represent means ±95% confidence interval of 7–13 fish. Means significantly ($p < 0.05$) different from control are indicated with an asterisk. Time is plotted on a log scale and actual values are shown.

TABLE 7. Effect of water pH on swimming time, buoyancy, internal pressure, coefficient of condition (*K*), and weight before the start (*B*) and after the termination (*A*) of the swimming performance experiment. Values represent means ± 95% confidence interval of 33–40 fish. **Significantly (*t*-test, $p < 0.001$) different from pH 7.7.

	pH 7.7	pH 5.3
Swimming time (h)	6.91±0.51	7.05±0.51
Buoyancy (mL·g⁻¹)	0.439±0.027	0.502±0.027**
Internal pressure (atm)	1.006±0.010	0.992±0.016
KB	0.986±0.008	0.988±0.013
KA	0.932±0.010	0.897±0.018**
Weight *B* (g)	2.74±0.10	2.80±0.10
Weight *A* (g)	2.60±0.10	2.55±0.10

inefficiency of hydrodynamic compensation (i.e. negative angle of attack) for transient excess lift (Alexander 1972), it is intriguing to speculate that the higher buoyancy of treated fish, which was of similar magnitude to that found in fish after 48 h in current of constant velocity, may have been responsible for the increased energy demands. Surprisingly the negative correlation found between buoyancy and the coefficient of condition after the termination of the experiment was not quite significant. Probably ventilation and osmoregulation (see below) contribute to increased energy expenditure under acid exposure. Neville (1979a) and Janssen and Randall (1975) did not find a long-term ventilatory response to reduced pH by rainbow trout. However, Rosseland (1980) reported such a response for brown trout (*Salmo trutta*). Treated fathead minnows displayed higher breathing rates (132 opercular beats·min^{-1}) compared with control fish (114 beats·min^{-1}) within 15 min of transfer from current into still water (Jansen 1986). This suggests that under severe exercise, when O_2 consumption is maximal, ventilation can be affected.

Mechanisms of Impaired Swimbladder Function

Effects of reduced environmental pH on the physiology of freshwater fish include disturbances in branchial gas exchange (Packer and Dunson 1972; Packer 1979; Ultsch et al. 1981), plasma acid–base status (Packer and Dunson 1970; Packer 1979; Neville 1979b; McDonald et al. 1980; Ultsch et al. 1981; Hobe et al. 1983; McDonald and Wood 1981; Booth et al. 1982), and haematological homeostasis (Dively et al. 1977; Neville 1979b; McWilliams 1982a; Milligan and Wood 1982; Giles et al. 1984). Of these factors, reduced O_2 uptake at the respiratory surface, and consequently decreased blood PO_2, which via an elevated O_2 gradient between swimbladder and blood (see below) potentially results in increased gas reabsorption, appears to arise only at acutely lethal pH values (2.0–3.5; Packer and Dunson 1972; Packer 1979; Ultsch et al. 1981). Similarly, net acid uptake can normally be avoided at ambient pH or 5.1 or lower (Ultsch et al. 1981), thus preventing significant changes in blood pH. However, fathead minnows are among the more acid-sensitive species (Rahel and Magnuson 1983; Mills 1982; Rahel 1984) and the possible development of a mild acidosis cannot be ignored even at a slightly more moderate pH.

Contrary to the first two factors, net acid uptake and impaired O_2 transport, an elevated haematocrit has been reported for acid-exposed fish over a wider pH range (4.0–6.4; McWilliams 1982a; Lee et al. 1983; Giles et al. 1984). Importantly, this increase in blood haematocrit is not linked to a parallel increase in blood haemoglobin content, but is partly attributable to simple swelling (increase in volume) of the erythrocytes (Milligan and Wood 1982, Giles et al. 1984). Milligan and Wood (1982) attributed a 68–125% increase in blood viscosity of rainbow trout directly to a concomitant rise in haematocrit and suggested an intimate linkage between ionic imbalance, elevated haematocrit, and increased blood viscosity. If this interpretation is correct, then, the strong correlation between moderately depressed environmental pH (4.0–6.0) and ionoregulatory failure (Leivestad and Muniz 1976; McDonald et al. 1980; McDonald and Wood 1981; McWilliams 1982a; Booth et al. 1982; Fraser and Harvey 1984; Giles et al. 1984) indicates that higher blood viscosity could be a common phenomenon at reduced ambient pH. Furthermore, unlike most natural situations, present experiments were conducted in moderately hard water which may have ameliorated the acid effect on ionoregulation (McDonald et al. 1980; McWilliams 1982b; Brown 1982) and consequently swimbladder performance.

Both gas secretion and reabsorption are critically dependent on the partial pressure gradient between the blood and gas phase, the gas (particularly O_2) combining capacity of the blood, and blood flow (Steen 1963a, 1963b; Kuhn and Kuhn 1961; Sund 1977). At a given partial pressure, the affinity of haemoglobin for O_2 and the O_2-carrying capacity of blood falls with declining blood pH (Bohr and Root effect). In the rete

mirabile of the swimbladder, where a localized reduction in blood pH is achieved by the addition of lactic acid (Hall 1924; Steen 1963b), these effects are used to produce high partial gas pressures. Arterial blood entering the rete at a reduced plasma pH and therefore lowered absolute O_2 content theoretically diminishes the effectiveness of the gas secreting mechanisms. In the reabsorptive area of the swimbladder, a reduced O_2 combining capacity of the afferent blood equally results in a depressed gas reabsorption rate. The Poiseuille equation (Poiseuille 1842, cited in Ross 1979b) predicts that blood flow rates inside vessels are, under otherwise constant conditions, inversely proportional to blood viscosity. Increases in heart rate and mean dorsal aortic pressure (Milligan and Wood 1982) indicate that acid-exposed salmonids do not compensate for higher blood viscosity by reducing vascular resistance. If this is true for fathead minnows, then the efficiency of gas secretion and reabsorption will be further reduced by the acid-induced increase in blood viscosity.

Ecological Implications

Rosseland et al. (1980) first referred to the "the higher costs of living in acid water." A limited capacity to efficiently regulate swimbladder lift represents a further "cost" component, at least in fathead minnows. Prolonged periods of gas secretion, hydrodynamical compensation of suboptimal buoyancy, and more frequent air gulping will increase maintenance requirements. These must be first met before any surplus energy can be allocated to growth or reproductive tissue (Kitchell 1983). Consequently, acid-exposed fish will funnel less of the available food into net energy gain. In times of food shortage, body energy reserves deplete more rapidly, as illustrated by the larger weight loss of unfed treated fish during sustained swimming. Since a decline in invertebrate fish prey (Almer et al. 1974; Hendry et al. 1976; Malley et al. 1981; Nero and Schindler 1983) and impaired chemoreception that potentially interfers with food selection and consumption in fathead minnows (Lemly and Smith 1985) have both been attributed to moderate decreases in environmental pH, these fish are likely under increasing pressure on both sides of the energy equation. Besides the metabolic effects, increased activity (Huey and Pianka 1981; Eggers 1982) and surface exposure may render fatheads more susceptible to predation. Furthermore, suboptimal buoyancy may reduce tolerance to maximal water velocities or the time to fatigue in current. If fish cannot withstand the flow of water, upstream dispersal will be limited and/or drift to unfavorable downstream habitats increased. Consequently, the viability of localized populations, spawning success, food availability, etc., may be negatively affected. All these factors potentially contribute to the disappearance or absence of cyprinids from aquatic ecosystems at pH levels of 5.2–6.2 as reported by Almer et al. (1974), Mills (1982), Rahel and Magnuson (1983), and Rahel (1984).

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Genetic Variation and Population Structure of Pacific Ocean Perch (*Sebastes alutus*)[1]

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Seeb, L. W., and D. R. Gunderson. 1988. Genetic variation and population structure of Pacific ocean perch (*Sebastes alutus*). Can. J. Fish Aquat. Sci. 45: 78–88.

We conducted an allozyme analysis of 27 collections representing over 1500 individuals of Pacific ocean perch (*Sebastes alutus*). Data were gathered from 25 presumptive loci of which 20% were polymorphic. Average observed heterozygosity was 0.069. Samples originated from throughout the commercially important range of the species from the Washington coast to the Bering Sea. Pacific ocean perch are highly similar throughout the range, with only a slight amount of population differentiation (F_{ST} = 0.023). However, a cline of gene frequencies within the Gulf of Alaska exists at *Ada2*, and there are significant frequency differences between the extremes of the range for several additional loci. There is no evidence of an interruption of gene flow between the western Gulf of Alaska and the Bering Sea. Stock delineations based on age structure, age–length relationships, and ages at maturity were not supported by the allozyme data and are either influenced by nongenetic environmental factors or are under a selective regime not measured by allozyme analyses.

Vingt-sept collections de sébaste à longue mâchoire (*Sebastes alutus*), totalisant 1 500 poissons, ont fait l'objet d'une analyse des allozymes. Des données ont été recueillies à 25 loci présomptifs dont 20 % étaient polymorphes. L'hétérozysotie moyenne observée s'élevait à 0,069. Les échantillons ont été recueillis dans toute l'aire de répartition de l'espèce faisant l'objet d'une exploitation commerciale importante de la côte de l'État de Washington à la mer de Béring. L'étude a révélé que l'espèce est très homogène dans toute son aire de répartition et qu'il n'existe qu'une faible différenciation démographique (F_{ST} = 0,023). Il existe toutefois un cline des fréquences géniques à *Ada2* chez les poissons du golfe de l'Alaska et des différences significatives de la fréquence à plusieurs autres loci entre les extrémités de l'aire de répartition. Les données ne prouvent pas qu'il y a eu interruption du flux génique entre la partie ouest du golfe de l'Alaska et la mer de Béring. La délimitation du stock basée sur la structure des âges, les relations âge-longueur et l'âge à la maturité n'a pas été étayée par les données sur les allozymes; cette délimitation du stock est soit touchée par des facteurs environnementaux non génétiques ou sous l'influence d'un régime de sélection non quantifié par les analyses des allozymes.

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Marine fishes, unlike many anadromous and freshwater fishes, often have very large spawning populations, and many have pelagic larvae. These factors contribute to a high potential for gene flow, and many species are relatively homogeneous over large geographic areas. Examples of homogeneity over a large geographic range can be found in the genera *Chanos* (Winans 1980), *Clupea* (Grant and Utter 1984; Grant 1984; Ryman et al. 1984), *Gadus* (Mork et al. 1985), *Hippoglossus* (Grant et al. 1984), *Theragra* (Grant and Utter 1980), and in several species of trans-Pacific shore fishes (Rosenblatt and Waples 1986). However, other marine species demonstrate intraspecific genetic differentiation (Shaklee 1983; Waples 1987). In addition, statistically significant allelic frequency differences have been found in several species sampled at the same locality in successive years (Kornfield et al. 1982; Gyllensten 1985). Thus, the life history of the fish including location and nature of spawning, duration of larval period, migrations of adults, and historical zoogeographic events must be considered when studying the amount of population differentiation and gene flow in marine species.

Pacific ocean perch (*Sebastes alutus*) were, in recent times, the most abundant rockfish species in the northeastern Pacific Ocean, ranging mainly offshore from central California to the Bering Sea at depths of 150–450 m. They are viviparous, releasing larvae that are about 6 mm long (Westrheim 1975; Efremenko and Lisovenko 1970). The larvae are pelagic, but adult migrations are largely confined to limited seasonal shifts in depth. Since their drastic decline in abundance in the 1960s, Pacific ocean perch have received considerable attention with studies concerning their biology (Westrheim 1973, 1975; Carlson and Haight 1976), stock distributions (Westrheim 1970, 1972, 1973, 1974, 1975; Gunderson 1972, 1977; Gunderson et al. 1977; Chikuni 1975), and population genetics (Johnson et al. 1970, 1971, 1973; Wishard et al. 1980).

Stocks of Pacific ocean perch have been delineated based on differential size distributions, year class strengths, and age–

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length relationships. Westrheim (1970, 1973) identified two stocks of Pacific ocean perch in the Northeast Pacific, tentatively categorizing these stocks as "British Columbia" and "Gulf of Alaska," with the dividing line near Dixon Entrance (lat. 54°40′N). Chikuni (1975) identified four distinct groups: an eastern Pacific stock distributed from British Columbia to California, a Gulf of Alaska stock which showed no large-scale intermixture with the eastern Pacific stock, an Aleutian stock which may receive larvae from the Gulf of Alaska, and a Bering Sea stock which probably receives larvae from both the Gulf of Alaska and the Aleutian stock.

Local subdivision on a lesser geographic scale has also been found. Gunderson (1972) identified considerable variation in mean length and growth of Pacific ocean perch from Queen Charlotte Sound and concluded that the Pacific ocean perch formed aggregations with different biological characteristics. In a later study, Gunderson et al. (1977) reported consistent differences in size and age composition between Moresby and two adjacent gullies, Goose Island and Mitchell's, in Queen Charlotte Sound.

We obtained Pacific ocean perch from throughout the commercially important range with collections from Washington, British Columbia (including Moresby, Goose Island, and Mitchell's gullies), the Gulf of Alaska, the Aleutian Islands, and the eastern Bering Sea. Our objectives were to assess the amount of allozymic variation over the range of Pacific ocean perch, to investigate genetic relationships within the Queen Charlotte Sound area (where local variation of life history characters has been reported), and to relate the levels of gene flow and geographic variation observed in Pacific ocean perch to other marine species with varying life history patterns. We found Pacific ocean perch to be highly similar throughout the range, although there are significant frequency differences at some loci between the extremes of the range.

Materials and Methods

Tissue samples for the electrophoretic analyses were collected from trawl-caught fish (Table 1; Fig. 1). Approximately 10 g each of liver and muscle tissue was extracted from each fish and frozen as soon as possible onboard ship. Tissues remained frozen until analysis. Twenty-seven collections of Pacific ocean perch were obtained totaling nearly 1500 individuals. Sampling included 2 collections from the Washington coast, 9 from British Columbia waters, 10 from the eastern Gulf of Alaska, 3 from the Kodiak Island area, and 1 from the Aleutian Islands. Fifty fish from each trawl were sampled if available and pooled for analysis. Pacific ocean perch catches were low in the Bering Sea, so samples from a number of trawls were pooled into a "northern" and a "southern" Bering Sea collection. Six of the British Columbia trawl hauls were selected for intergully comparisons. These originated from Goose Island, Moresby, and Mitchell's gullies of Queen Charlotte Sound.

We screened for allozyme activity at a large number of enzyme systems (e.g. Seeb et al. 1987) using techniques described by Harris and Hopkinson (1976) and May et al. (1979). Three buffer systems were used to resolve 25 loci: (I) a Tris–citrate gel and tray buffer (pH 7.0) (Shaw and Prasad 1970); (II) an amine–citrate buffer described by Clayton and Tretiak (1972) (pH 6.5); and (III) a Tris – citric acid gel buffer (pH 8.5), lithium hydroxide – boric acid tray buffer (pH 8.5) (Ridgway et al. 1970). Locus and allele designations followed that outlined in Wishard et al. (1980) and the conventions of Allendorf et al. (1983) (Table 2).

Variation among collections was quantified using three statistical procedures. Nei's genetic identity (I) (Nei 1972) was used to assess the overall similarity in allelic composition and frequency between sample collection sites. A matrix of (I) values between all pairs of collections was generated and used to construct a phenogram using the unweighted pair group method with arithmetic averages (UPGMA) (Sneath and Sokal 1973). The degree of genetic structuring and differentiation as measured by F-statistics (Wright 1965; Nei 1977) was calculated for each locus over all collections. A third procedure, discriminant analysis (SPSS direct procedure; Nie et al. 1975), was used to describe the functions that maximize separation between collection sites and identify the loci contributing significantly to their separation. At each locus the number of discriminating variables was equivalent to the number of alleles minus 1, with the most common allele being the one omitted. Each fish was scored according to the number of doses of particular alleles.

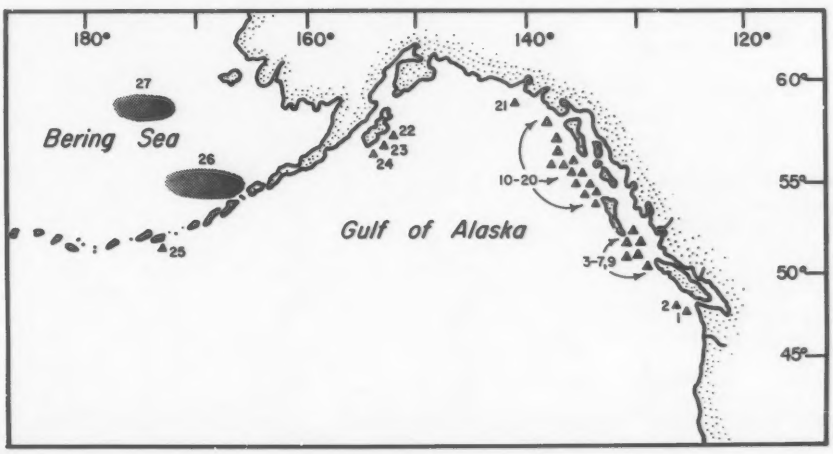

FIG. 1. Collection locations of all samples analyzed in this study. Samples 26 and 27 were combined from a number of separate locations within the shaded areas for the analysis.

TABLE 1. Description of the Pacific ocean perch samples analyzed in this study.

Sample No.	*N*	Depth (m)	Lat. N	Long. W	Approximate location
1	50	207	48°11′	124°56′	Washington coast
2	51	265	48°17′	125°04′	Washington coast
3	51	179	51°11′	128°35′	Goose Island Gully
4	50	274	51°20′	129°20′	Goose Island Gully
5	50	347	51°42′	129°57′	Mitchell's Gully
6	50	201	51°54′	129°20′	Mitchell's Gully
7	50	201	52°59′	130°19′	Moresby Gully
8	50	—	—	—	British Columbia
9	50	347	52°00′	130°19′	Moresby Gully
10	50	420	54°10′	133°23′	Off Langara Island
11	50	256	54°03′	133°34′	Off Frederick Island
12	21	240	54°56′	134°19′	Dixon Entrance
13	50	179	55°18′	134°13′	Dixon Entrance
14	37	310	56°06′	134°55′	Chatham Strait
15	48	192	56°10′	134°59′	Chatham Strait
16	50	333	56°07′	135°13′	Off Baranof Island
17	49	223	56°23′	135°36′	Off Baranof Island
18	50	230	56°33′	135°43′	Off Baranof Island
19	51	157	57°41′	136°35′	Off Chichagof Island
20	50	355	58°01′	136°45′	North of Chichagof Island
21	30	338	59°21′	141°24′	Off Yakutat
22	50	199	56°17′	152°44′	Off Kodiak Island
23	35	245	55°55′	154°11′	Off Kodiak Island
24	46	192	55°55′	154°12′	Off Kodiak Island
25	98	256	52°18′	174°47′	Aleutian Islands
26	56	182–731	54°36′–56°38′	165°31′–173°09′	S. Bering Sea
27	161	182–215	57°48′–58°58′	173°43′–178°11′	N. Bering Sea

TABLE 2. Enzymes, enzyme abbreviations, Enzyme Commission numbers, and the preferred buffers and tissues used in this study. Designation of buffers are I (Shaw and Prasad 1970), II (a modification of Clayton and Tretiak 1972), and III (Ridgway et al. 1970). L = liver; M = muscle.

Enzyme	E.C. No.	Abbreviation	Buffer	Tissue
β-*N*-acetylgalactosaminidase	3.2.1.53	*bGala*	II	L
Acid phosphatase	3.1.3.2	*Acp*	II	L
Adenosine deaminase	3.5.4.4	*Ada1*	I	M
		Ada2	I	M
Adenylate kinase	2.7.4.3	*Ak*	II	M
Alcohol dehydrogenase	1.1.1.1	*Adh*	II	L
Creatine kinase	2.7.3.2	*Ck*	III	M
Dipeptidase[a]	3.4.13.11	*Dpep*	I	M
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi1*	III	M
		Gpi2	III	M
β-Glucuronidase	3.2.1.31	*bGus*	III	L
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gapdh1*	II	M
		Gapdh2	II	M
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*Gpdh*	I	M
Hydroxyacylglutathione hydrolase	3.1.2.6	*Hgh*	III	L
Isocitrate dehydrogenase	1.1.1.42	*Idh1*	II	M
		Idh2	II	L
Lactate dehydrogenase	1.1.1.27	*Ldh*	II	L
Malate dehydrogenase	1.1.1.37	*Mdh1*	II	M
		Mdh2	II	M
Mannose-6-phosphate isomerase	5.3.1.8	*Mpi*	II	M
Phosphoglucomutase	5.4.2.2	*Pgm*	III	M
Phosphogluconate dehydrogenase	1.1.1.44	*Pgdh*	II	M
Superoxide dismutase	1.15.1.1	*Sod2*	II	M
		Sod3	III	L

[a]Resolved with DL-leucyl-DL-alanine.

Seven polymorphic loci were included in the analysis (*Gpdh, Pgm, Gpi2, Ada2, Pgdh, Idh2,* and *Adh*) producing 16 discriminating variables. Standardized discriminant function coefficients were calculated for each significant function. The absolute value of these coefficients can be interpreted as the relative contribution of each variable to the discriminant function. A centroid (group mean score on each function) was calculated by averaging the scores for each fish within a particular collection. These can be plotted for each group with n significant functions in n-dimensional space.

Heterogeneity of gene frequencies within and among the six collections taken from Queen Charlotte Sound was tested with a log-likelihood analysis (Smouse and Ward 1978) using the G-statistic of Sokal and Rohlf (1981). The analysis was designed in a nested form, with total heterogeneity partitioned into three gullies (Mitchell's, Moresby, and Goose Island), and followed the procedures outlined in Wishard et al. (1984). Each gully was further divided to allow comparisons between collections taken within the gullies.

Estimates of gametic (linkage) disequilibrium in the sample collections and in the total population were calculated following the methods of Cockerham and Weir (1977) and Weir (1979) using the FORTRAN program "Linkdis" (Black and Krafsur 1985). Alleles were pooled into two classes (one comprising only the most frequent allele, and the other all remaining alleles).

Results

Variation within Collection Sites

Twenty-five presumptive electrophoretic loci were successfully resolved in each of the collections. Of the 25 loci, five (20%) were polymorphic at the 0.05 level or greater in at least one collection. An additional nine (36%) loci had occasional variants occurring between 0.01 and 0.05 in at least one collection (Table 3). Significant deviations from the Hardy–Weinberg expectation occurred in 9 (5%) of the 173 tests performed, equivalent to what could be expected by chance alone. Average expected heterozygosity values were calculated for each collection and ranged between 0.060 and 0.076. The mean value for all collections was 0.069. This value is similar to the mean value calculated for 51 species of fish by Nevo (1978) (H_{exp} = 0.051) and for 106 species of marine fish by Smith and Fujio (1982) (H_{exp} = 0.055).

Variation among Collection Sites

Local geographic variation among the three gullies in Queen Charlotte Sound was evaluated with a hierarchical log-likelihood analysis of six collections. Two were available from each of Mitchell's Gully (5, 6), Moresby Gully (7, 9), and Goose Island Gully (3, 4). The analysis was based on eight loci (*Gpdh, Pgm, Gpi2, Pgdh, Adh, Idh2, Ada2,* and *Acp*) (Table 4). No significant differences ($P < 0.05$) were detected either among gullies or within gullies in comparisons at the eight loci (Table 4).

Geographic variation over the entire range was evident at some loci. The *Gpi2(87)* allele occured in two British Columbia collections, two collections from the eastern Gulf of Alaska, and in all Aleutian and Bering Sea collections. Similarly, *Adh(66)* increased in frequency in the western part of the range, particularly in the Kodiak Island and southern Bering Sea collections. *Ada2* was also highly polymorphic, and the frequency of *Ada2(100)* varied from approximately 0.67 off the Washington coast to approximately 0.87 in the western Gulf of Alaska and the eastern Bering Sea. The regression of gene frequency and latitude was significant ($P < 0.001$, arcsine transformation). These differences indicate some population structuring.

Two other loci were also highly variable, but we detected no consistent pattern of variation. The frequency of *Gpdh(100)* ranged from 0.415 to 0.700; the frequency of *Pgm(100)* ranged from 0.360 to 0.622. No significant regressions with depth or latitude were found for either locus. We tested for gametic disequilibrium between *Pgm* and *Gpdh* in all sample collections and in the total populations. Significant gametic disequilibrium was found in only collection 17 (chi-square = 3.92, 1 df, P = 0.0476).

The identity values (I) between each pair of collections ranged between 0.9935 and 0.9999, indicating a very high degree of overall similarity between the collections. The matrix of all pairwise values (available from the authors) were used to construct a phenogram (Fig. 2), and the results showed a very tight clustering, with all collections joining at the 0.997 level. Some structuring is suggested in the phenogram, but it must be viewed in context of the high identity values. Phenograms constructed with Nei's identity (I) values for populations of a single species may possess relatively large standard errors. Under these constraints, it appeared that the Washington coast collections were closely related to each other and belonged to a larger grouping including the majority of the British Columbia and eastern Gulf of Alaska collections. At the 0.9973 level, this group is joined by a second group including the remainder of the eastern Gulf of Alaska collections, and the collections from the Aleutian Islands and northern Bering Sea. This second cluster seemed to have no obvious geographic basis, and may have represented the high frequency (>0.60) of the *Gpdh(100)* allele. A third and final group included the southern Bering Sea collection and all three Kodiak Island collections.

The low level of genetic divergence between collection sites is also seen in the F_{ST} values. The F_{ST} values were calculated for each locus and ranged from 0.0006 to 0.0429 (average = 0.0229).

A discriminant analysis, which can reveal population structuring not apparent by other methods (Yeh et al. 1985), was conducted on the 23 collections with complete data sets. Two significant discriminant functions explained 55% of the variation. The first explained 35% of the variation ($P < 0.0001$) and the second an additional 20% ($P < 0.001$). The standardized discriminant function coefficients for all of the variables used in the analysis are given in Table 5, and those with the largest absolute values were the most important in group discrimination. *Ada2(70), Adh(66),* and *Gpi2(87),* in that order, contributed most heavily to Function 1. The most important variables contributing to Function 2 were, in descending order of importance *Ada2(70), Pgdh(105),* and *Idh2(68).*

The group centroids for Function 1 and Function 2 (Fig. 3) showed that collections 1–19 (except 3, 14, and 18), originating from the Washington coast to Chichagof Island, were closely grouped, with their centroids having positive values for both functions. Samples 3, 14, and 18 are intermediate between the two positive and negative groups. Samples 20–27 (except 26) have negative values for both functions. Sample 26 (southern Bering Sea) was distinct from all other collections. It has extreme frequencies for nearly all the alleles contributing heavily to each discriminant function. The dissimilarity of collection 26 was not identified by the clustering technique.

TABLE 3. Allele frequency estimates for Pacific ocean perch (*Sebastes alutus*).

Collection No.	*Acp*			*Ada2*			*Adh*					*Gpi1*		
	100	56	145	100	70	60	100	66	46	110	164	100	66	300
1	1.000	—	—	0.670	0.330	—	0.940	0.060	—	—	—	1.000	—	—
2	0.979	0.021	—	0.676	0.324	—	0.980	0.020	—	—	—	1.000	—	—
3	0.990	0.010	—	0.653	0.347	—	1.000	—	—	—	—	1.000	—	—
4	0.990	0.010	—	0.635	0.365	—	0.970	0.030	—	—	—	1.000	—	—
5	1.000	—	—	0.673	0.327	—	0.976	0.024	—	—	—	1.000	—	—
6	0.980	0.020	—	0.663	0.337	—	0.978	0.022	—	—	—	1.000	—	—
7	0.989	0.011	—	0.684	0.316	—	0.983	0.017	—	—	—	1.000	—	—
8	1.000	—	—	0.720	0.280	—	0.960	0.020	—	—	0.020	1.000	—	—
9	1.000	—	—	0.640	0.360	—	0.968	0.032	—	—	—	1.000	—	—
10	0.979	0.021	—	0.729	0.271	—	0.987	0.012	—	—	—	1.000	—	—
11	0.990	0.010	—	0.720	0.280	—	1.000	—	—	—	—	1.000	—	—
12			—	0.786	0.214	—	0.976	0.024	—	—	—	1.000	—	—
13			—	0.847	0.153	—	0.990	—	0.010	—	—	1.000	—	—
14	1.000	—	—	0.757	0.243	—	0.960	0.027	0.013	—	—	1.000	—	—
15	0.990	0.010	—	0.772	0.228	—	1.000	—	—	—	—	1.000	—	—
16							0.959	0.041	—	—	—	1.000	—	—
17				0.850	0.150	—	0.959	0.041	—	—	—	1.000	—	—
18	0.969	0.031	—	0.850	0.150	—	0.979	0.021	—	—	—	1.000	—	—
19	1.000	—	—	0.740	0.260	—	0.980	0.020	—	—	—	1.000	—	—
20	1.000	—	—	0.870	0.130	—	0.919	0.070	0.011	—	—	1.000	—	—
21	1.000	—	—	0.931	0.069	—	0.929	0.071	—	—	—	1.000	—	—
22	0.990	0.010	—	0.878	0.122	—	0.880	0.120	—	—	—	0.990	0.010	—
23	0.985	0.015	—	0.857	0.114	0.029	0.850	0.150	—	—	—	1.000	—	—
24	0.956	0.033	0.011	0.889	0.111	—	0.844	0.156	—	—	—	1.000	—	—
25	0.995	0.005	—	0.866	0.134	—	0.953	0.042	—	0.005	—	0.995	—	0.005
26	0.991	0.009	—	0.875	0.125	—	0.867	0.123	—	—	0.009	1.000	—	—
27	0.997	0.003	—	0.832	0.168	—	0.953	0.047	—	—	—	1.000	—	—

	Gpi2			*Gpdh*			*Idh1*			
300	100	87	105	100	66	138	100	80	124	110
—	1.000	—	—	0.592	0.408	—	1.000	—	—	—
—	1.000	—	—	0.559	0.441	—	1.000	—	—	—
—	1.000	—	—	0.539	0.461	—	1.000	—	—	—
—	0.990	0.010	—	0.500	0.500	—	1.000	—	—	—
—	1.000	—	—	0.589	0.411	—	1.000	—	—	—
—	0.990	0.010	—	0.573	0.427	—	0.990	—	0.010	—
—	1.000	—	—	0.500	0.490	0.010	1.000	—	—	—
—	1.000	—	—	0.490	0.510	—	1.000	—	—	—
—	1.000	—	—	0.570	0.430	—	1.000	—	—	—
—	1.000	—	—	0.622	0.378	—	1.000	—	—	—
—	1.000	—	—	0.550	0.450	—	1.000	—	—	—
—	1.000	—	—	0.548	0.452	—	1.000	—	—	—
—	1.000	—	—	0.510	0.490	—	0.990	0.010	—	—
—	0.986	—	0.014	0.635	0.365	—	1.000	—	—	—
—	1.000	—	—	0.415	0.585	—	1.000	—	—	—
—	1.000	—	—	0.700	0.300	—	1.000	—	—	—
—	1.000	—	—	0.480	0.520	—	1.000	—	—	—
—	0.960	0.040	—	0.592	0.408	—	1.000	—	—	—
—	1.000	—	—	0.592	0.408	—	1.000	—	—	—
—	1.000	—	—	0.690	0.310	—	0.990	0.010	—	—
—	0.983	0.017	—	0.667	0.333	—	0.983	—	—	0.017
—	1.000	—	—	0.510	0.490	—	1.000	—	—	—
—	1.000	—	—	0.500	0.500	—	1.000	—	—	—
—	0.978	0.011	0.011	0.446	0.554	—	1.000	—	—	—
0.005	0.990	0.005	0.005	0.655	0.345	—	1.000	—	—	—
—	0.973	0.027	—	0.464	0.536	—	1.000	—	—	—
—	0.988	0.006	0.006	0.608	0.392	—	1.000	—	—	—

TABLE 3. (*Concluded*)

Collection No.	*Idh2*					*Mdh2*			*Mpi*		*Pgm*		
	100	110	80	68	120	100	75	124	100	160	100	77	62
1	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.510	0.490	—
2	0.990	0.010	—	—	—	1.000	—	—	1.000	—	0.510	0.490	—
3	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.431	0.569	—
4	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.490	0.510	—
5	0.987	—	—	0.013	—	1.000	—	—	1.000	—	0.510	0.490	—
6	1.000	—	—	—	—	1.000	—	—	1.000	—	0.390	0.610	—
7	1.000	—	—	—	—	1.000	—	—	1.000	—	0.532	0.468	—
8	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.440	0.560	—
9	0.988	—	—	0.012	—	0.990	—	0.010	1.000	—	0.480	0.520	—
10	1.000	—	—	—	—	1.000	—	—	1.000	—	0.622	0.378	—
11	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.450	0.550	—
12	1.000	—	—	—	—	1.000	—	—	1.000	—	0.381	0.595	0.0
13	0.980	—	0.020	—	—	1.000	—	—	1.000	—	0.460	0.540	—
14	0.986	—	—	0.014	—	1.000	—	—	1.000	—	0.487	0.513	—
15	1.000	—	—	—	—	1.000	—	—	1.000	—	0.418	0.582	—
16	1.000	—	—	—	—	1.000	—	—	1.000	—	0.550	0.450	—
17	1.000	—	—	—	—	1.000	—	—	1.000	—	0.375	0.625	—
18	1.000	—	—	—	—	1.000	—	—	1.000	—	0.360	0.640	—
19	1.000	—	—	—	—	1.000	—	—	1.000	—	0.490	0.510	—
20	0.970	—	—	0.030	—	0.990	0.010	—	0.990	0.010	0.490	0.510	—
21	0.965	—	—	0.035	—	1.000	—	—	1.000	—	0.569	0.431	—
22	0.990	—	—	0.010	—	1.000	—	—	1.000	—	0.490	0.510	—
23	0.960	—	—	0.040	—	1.000	—	—	1.000	—	0.397	0.603	—
24	0.987	—	—	0.013	—	1.000	—	—	1.000	—	0.533	0.467	—
25	0.944	—	—	0.051	0.005	0.995	0.005	—	1.000	—	0.515	0.485	—
26	0.991	—	—	0.009	—	1.000	—	—	1.000	—	0.366	0.634	—
27	0.966	—	—	0.034	—	1.000	—	—	1.000	—	0.490	0.510	—

m		*Pgdh*				*Sod2*		*Sod3*	
7	62	100	95	105	80	100	161	100	60
90	—	0.990	0.010	—	—	1.000	—	1.000	—
90	—	0.971	0.019	0.010	—	1.000	—	1.000	—
69	—	0.970	0.020	—	0.010	1.000	—	0.990	0.010
10	—	1.000	—	—	—	1.000	—	1.000	—
90	—	0.980	0.020	—	—	1.000	—	1.000	—
10	—	0.980	0.020	—	—	0.990	0.010	1.000	—
68	—	0.989	0.011	—	—	1.000	—	1.000	—
60	—	1.000	—	—	—	1.000	—	1.000	—
20	—	0.970	0.030	—	—	1.000	—	1.000	—
78	—	0.980	0.020	—	—	1.000	—	1.000	—
50	—	0.980	0.020	—	—	1.000	—	1.000	—
95	0.024	0.976	0.024	—	—	1.000	—	1.000	—
40	—	1.000	—	—	—	1.000	—	1.000	—
13	—	0.973	0.013	0.013	—	1.000	—	1.000	—
82	—	0.990	0.010	—	—	1.000	—	1.000	—
50	—	0.990	—	—	—	1.000	—	1.000	—
25	—	0.990	0.010	—	—	1.000	—	1.000	—
40	—	0.990	0.010	—	—	1.000	—	1.000	—
10	—	0.990	—	0.010	—	1.000	—	1.000	—
10	—	1.000	—	—	—	1.000	—	1.000	—
31	—	1.000	—	—	—	1.000	—	1.000	—
10	—	0.990	0.010	—	—	1.000	—	1.000	—
03	—	1.000	—	—	—	1.000	—	1.000	—
67	—	1.000	—	—	—	1.000	—	1.000	—
85	—	0.995	0.005	—	—	1.000	—	1.000	—
34	—	1.000	—	—	—	1.000	—	1.000	—
10	—	0.991	0.009	—	—	1.000	—	1.000	—

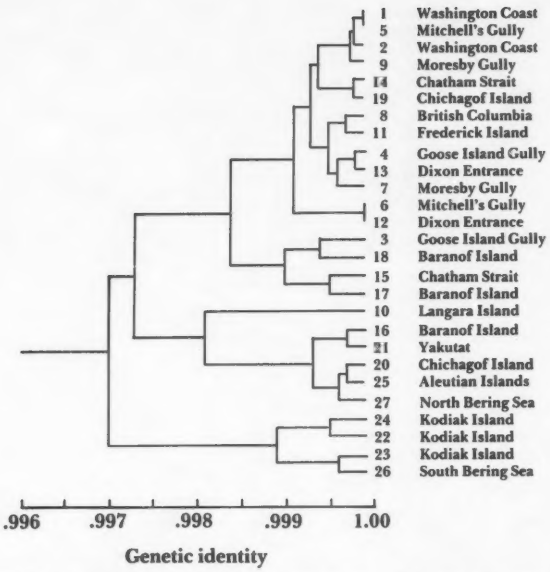

FIG. 2. Phenogram generated by UPGMA (Sneath and Sokal 1973) clustering techniques using Nei (1972) *I* values. The relationships between samples of Pacific ocean perch are shown.

Discussion

Genetic Differentiation

Pacific ocean perch are genetically quite similar throughout their range. They do not form discrete genetic stock groups, but show a gradual differentiation from Washington State to the Bering Sea consistent with an isolation by distance model. There are significant differences between extremes of the range at *Ada2* and in the distribution of rare alleles, but adjacent groups are similar. There is no indication that the area around Dixon Entrance, as described by Westrheim (1970, 1973), forms a barrier or dividing line between genetic stocks of perch. Furthermore, we could not detect an interruption of gene flow between the eastern Gulf of Alaska and the Bering Sea through the Aleutian Island chain such as occurs in Pacific herring (*Clupea harengus pallasi*) (Grant and Utter 1984).

Our results are in concordance with those of Quast (1987) who examined 18 morphometric characters in Pacific ocean perch collections and found that these characters also demonstrated clinal geographic variation in the region between Vancouver Island and the Bering Sea. He also found little evidence to support Barsukov's (1964) conclusion that two separate subspecies of Pacific ocean perch inhabit the areas north and south of the Aleutian arc.

The low levels of differentiation between collections are reflected in all three statistical analyses. The F_{ST} value of 0.0229 calculated for Pacific ocean perch is a relatively low value for marine fish, indicating little population differentiation. Winans (1980) calculated F_{ST} values for six species of marine fishes. These values ranged from 0.0041 for plaice (*Pleuronectes platessa*) to 0.3542 for silversides (*Menidia beryllina*). The high value calculated for *Menidia* is probably due to its life history characteristic of laying demersally attached eggs (Winans 1980). Waples (1987) calculated similar values for 10 species of marine shore fishes. Those values ranged from less than 0 to 0.373.

The cluster analysis also indicated a very high degree of similarity among populations. All collections clustered at the 0.997 level. Some geographic patterns were identified despite the contribution of several highly polymorphic loci that showed no obvious relationships to geography or depth. All collections from the western Gulf of Alaska, Aleutian Islands, and Bering Sea occurred outside of the first major grouping.

A similar relationship was suggested by the discriminant analysis. In this case, only 55% of the variation could be explained by the two functions. The plot of the group centroids suggested that the collections were subdivided along a continuum, with no abrupt disjunctions. The extreme frequencies for the most important variables of each discriminant function explain the position of collection 26. Neither *Pgm* nor *Gpdh* was heavily weighted by either function.

The distribution of *Pgm* and *Gpdh* frequencies did not conform to any geographic pattern. Johnson et al. (1971) reported evidence for a nonrandom distribution of *Pgm* phenotype with respect to depth, sex, and *Gpdh* phenotype. H. Tsuyuki (Department of Fisheries and Oceans, Vancouver, B.C. V6T 1X2, unpubl. data) observed *Gpdh* frequency variation associated with depth and age in repeated samplings from British Columbia. However, in this study, no significant regression equations were found with allelic frequency and the tested geographic variables. In addition, no consistent excesses or deficiencies of heterozygotes were observed at either locus indicative of admixtures of breeding units. Significant gametic disequilibrium was found in only 1 of 27 sample collections and was not detected in the total population. Age data are not available to test for relationships between age and genotype or age and overall heterozygosity.

The apparent cline in *Ada2* frequencies ranging from approximately 0.67 off the Washington coast to 0.87 in the western Gulf of Alaska and the Bering Sea is of particular interest. Clines may develop as a result of secondary contact of formerly disjunct differentiated populations or spatial differences in the selective environments of continuous populations. However, distinguishing among the major causes can be very difficult (Endler 1977).

In the first case, the disjunct populations could have differentiated either adaptively or by chance effects such as genetic drift, random effects of dispersal, or recurrent mutations in certain areas. In the second case, differential selective values could occur along environmental gradients or along abrupt spatial changes. Clines in several other fish species have been attributed to differential environmental temperatures found in various regions (Nevo and Bar 1976; Johnson 1977; Powers and Place 1978; Phillip et al. 1981). As Pacific ocean perch are widely distributed, adaptive differences of the alleles over a heterogeneous environment could be maintaining the cline.

Gene Flow in Pacific Ocean Perch

Pacific ocean perch vary geographically in life history characters, and for management purposes, stocks have been identified using such criteria as age at maturity, age–length relationships, fecundity, etc. (Table 6). Gunderson et al. (1977) showed that a distinct stock occurs in Moresby Gully in Queen Charlotte Sound, based on fork length and age distribution. There appears to be sufficient gene flow between Moresby and the other gullies to prevent measurable genetic differentiation while at the same time an insufficient amount of adult migration to counteract local differences in length and age composition.

TABLE 4. Log-likelihood analysis used to test for heterogeneity within and among Pacific ocean perch samples from gullies of Queen Charlotte Sound. *G* values are given for each locus and for all loci combined.

Source of variation	Acp		Ada2		Adh		Idh2	
	df	*G*	df	*G*	df	*G*	df	*G*
Among Gullies	2	0.4009	2	0.2656	2	0.5458	2	0.2991
Within Gullies	3	4.2888	3	0.5109	3	4.4445	3	2.9524
Mitchell's	1	2.8340	1	0.0230	1	0.0049	1	1.5838
Moresby	1	1.4546	1	0.4220	1	0.3572	1	1.3678
Goose Island	1	0.0002	1	0.0659	1	4.0824	1	0.0008
Total	5	4.6897	5	0.7765	5	4.9903	5	3.2515

Source of variation	Gpdh		Gpi2		Pgdh		Pgm	
	df	*G*	df	*G*	df	*G*	df	*G*
Among Gullies	4	3.6524	2	1.6186	2	0.9615	2	1.3654
Within Gullies	6	2.5537	3	2.8026	3	3.6778	3	4.1098
Mitchell's	2	0.0487	1	1.3913	1	0.0004	1	2.8970
Moresby	2	2.2453	1	0.0000	1	0.8641	1	0.5257
Goose Island	2	0.2597	1	1.4113	1	2.8133	1	0.6871
Total	10	6.2061	5	4.4212	5	4.6393	5	5.4752

Source of variation	df	Sum over all loci	Standardized *G*/df
Among Gullies	18	9.1093	0.5061
Within Gullies	27	25.3405	0.9385
Mitchell's	9	8.7831	0.9759
Moresby	9	7.2367	0.8041
Goose Island	9	9.3207	1.0356
Total	45	34.4498	0.7656

TABLE 5. Standardized discriminant function coefficients for each allele used as a variable in the discriminant analysis.

Variable	Standardized discriminant function coefficients	
	Function 1	Function 2
Gpdh(100)	0.241	−0.277
Pgm(77)	−0.169	0.204
Pgm(62)	0.054	−0.005
Gpi2(87)	−0.411	−0.020
Gpi2(105)	−0.070	−0.104
Pgdh(95)	0.160	0.029
Pdgh(105)	−0.236	0.524
Adh(66)	−0.453	−0.066
Adh(46)	−0.049	−0.116
Adh(110)	0.030	−0.193
Adh(164)	−0.138	0.201
Idh2(110)	0.040	0.085
Idh2(68)	−0.088	−0.415
Idh2(120)	0.042	−0.147
Ada2(70)	0.577	0.551

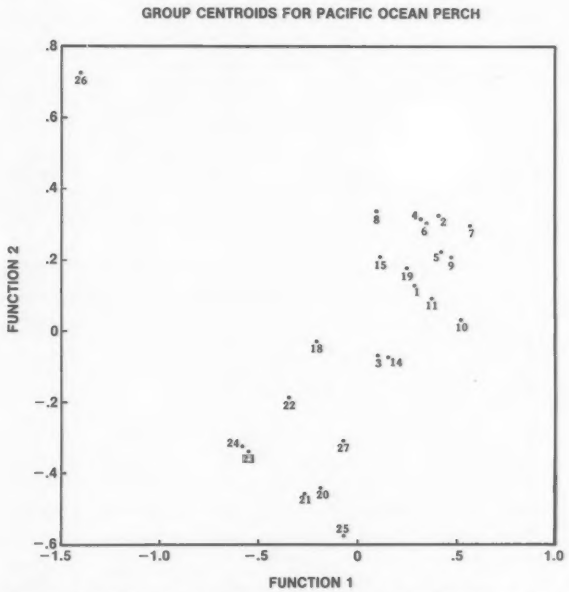

FIG. 3. Plot of the group centroids derived in the discriminant analysis of Pacific ocean perch.

These local differences probably reflect historical and environmental influences as well as fishing pressure.

Gene flow in Pacific ocean perch most likely occurs during the pelagic larval period. Larvae of Pacific ocean perch are released into the plankton in April and May in southeastern Alaska (Westrheim 1975) and drift with the currents until early summer. They probably take up a shallow demersal existence during the first year of life (Carlson and Haight 1976). As the fish grow older, they gradually move deeper. Their life history is presumably similar throughout the range, although peak parturition occurs during March in Queen Charlotte Sound and Washington (Gunderson 1977).

TABLE 6. Age fecundity schedules for Pacific ocean perch from the Washington – Vancouver Island area, Queen Charlotte Sound, and western Gulf of Alaska.

Age	Washington – Vancouver Island[a]		Queen Charlotte Sound[a]		Western Gulf of Alaska	
	Length (cm)	Fecundity (thousands)	Length (cm)	Fecundity (thousands)	Length (cm)[b]	Fecundity (thousands)[c]
9	32.9	17.8	34.3	Immature	27.1	Immature
10	34.3	25.1	35.9	Immature	28.6	11.5
11	35.5	34.0	37.2	45.7	29.9	14.6
12	36.6	43.7	38.5	55.7	31.1	17.9
13	37.6	54.7	39.6	67.3	32.2	21.6
14	38.6	66.6	40.6	78.6	33.1	25.0
15	39.4	80.8	41.4	90.1	34.0	28.8
16	40.2	93.9	42.2	100.4	34.8	32.6
17	41.0	108.8	42.9	111.5	35.5	36.2
18	41.6	125.7	43.6	122.1	36.1	39.6
19	42.2	139.8	44.1	133.5	36.7	43.2
20	42.7	155.2	44.6	142.2	37.2	46.4
21	43.2	169.2	45.0	151.3	37.7	49.8
22	43.7	184.3	45.4	158.9		
23	44.1	200.5	45.8	166.9		
24	44.5	214.3	46.1	175.2		

[a]From Gunderson (1977); length data are for females.
[b]From Westrheim (1973); length data are for females.
[c]From Lisovenko (1965); age at maturity from Westrheim (1975).

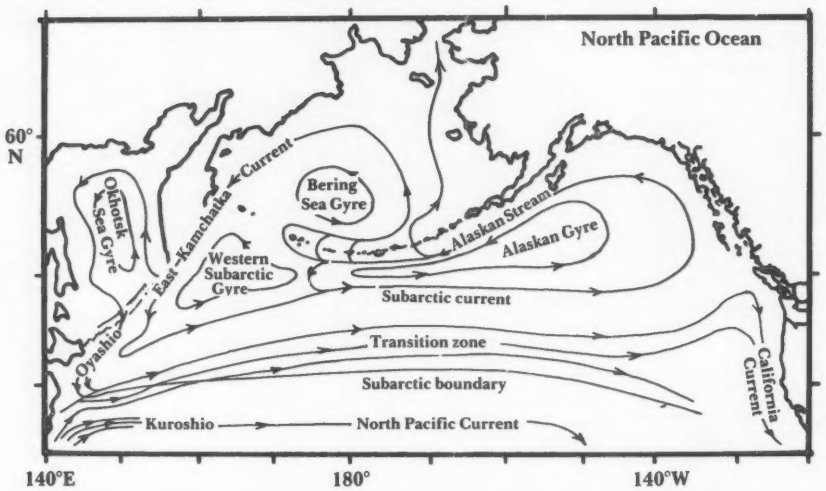

FIG. 4. Schematic presentation of major oceanographic features of the northern Pacific Ocean (after Favorite et al. 1977).

The prevailing ocean currents in the North Pacific were reviewed by Favorite et al. (1977) (Fig. 4). There is a northward flow around the Gulf of Alaska turning westward along the Alaska coast, with some northward flow through the Aleutian Islands. Some of the water discharges through the Bering Strait, but most continues westward around the Bering Sea basin and southward along the east coast of Kamchatka, completing the gyre. The prevailing currents branch into northward and southward components as they approach the British Columbia coast, and the location of this transition zone varies annually (Dodimead et al. 1963; Favorite et al. 1976). Thus, larvae released off Vancouver Island could drift in either direction.

Favorite et al. (1977) suggested that there is a northward transport of Pacific ocean perch larvae and that stocks along the west coast of the United States are replenished either by migration of adults or transport of larvae around the Gulf of Alaska gyre. Using an estimate of 1 yr for the pelagic period, they calculated that there is an adequate period to transport larvae around the gyre. However, our data do not indicate that there is a continuous gene flow of Pacific ocean perch around the Alaska gyre, and the pelagic period is probably shorter than that suggested by Favorite et al. (Carlson and Haight 1976).

Pacific ocean perch are genetically similar throughout the range, but do show a gradual differentiation from Washington State to the Bering Sea. The pelagic larval stage and prevailing

ocean currents seem to prevent any sharp genetic differentiation. Clearly, however, local aggregations of perch develop different age structures, age–length relationships, length–fecundity relationships, and ages at maturity. These life history characters are either influenced by nongenetic environmental and historical factors or, alternatively, are under a selective regime not measured by electrophoretic variants (Turner et al. 1979). Management schemes should consider both the underlying genetic structure as well as the measurable life history characters in developing strategies for successfully rebuilding the Pacific ocean perch resource.

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Significance of the Functional Response of Predators to Changes in Prey Abundance in Multispecies Virtual Population Analysis

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Hildén, M. 1988. Significance of the functional response of predators to changes in prey abundance in multispecies virtual population analysis. Can. J. Fish. Aquat. Sci. 45: 89–96.

Recent applications of multispecies assessment methods (MSVPA) to North Sea Fish stocks indicate that MSVPA and single-species assessments give highly correlated recruitment estimates. This may be an artifact due to the choice of the relationship between predation mortality and prey cohort size. If the predation mortality is an increasing function of prey cohort size over a certain range of cohort sizes, even a constant predator biomass may cause fluctuations in the number of fish recruiting to the fishable part of the population. Thus the correlation between single-species and multispecies recruitment estimates may be lower than earlier analyses have suggested, and changes in predation mortality may have caused fluctuations in year class strengths which previously have been attributed to unspecified environmental conditions. Because a relatively minor alteration of the predation function can cause such a drastic change in the perceived dynamics of the fish stocks, I suggest that more studies of the predation processes in fish communities are needed before one can hope to gain deeper insights into the recruitment of fish stocks by applying multispecies assessment methods.

Des applications récentes des méthodes d'évaluation multispécifiques (AVMSP) aux stocks de poissons de la mer du Nord montrent que les estimations du recrutement obtenues à partir des méthodes d'évaluation virtuelle multispécifique et monospécifique sont fortement corrélées. Il pourrait s'agir d'un artefact attribuable au choix de la relation entre la mortalité par prédation et la taille des cohortes de proies. Si la mortalité par prédation augmente en fonction de la taille des cohortes de proies dans un certain intervalle de tailles de cohortes, même une biomasse de prédateurs constante peut entraîner des fluctuations du nombre de recrues de la partie exploitable de la population. La corrélation entre les estimations du recrutement obtenues par les méthodes monospécifique et multispécifique pourrait donc être plus faible que ce que les analyses antérieures ont indiqué, et des changements de la mortalité par prédation pourraient avoir causé des fluctuations dans l'abondance des classes d'âge, précédemment attirbuées à des conditions environnementales non précisées. Étant donné qu'une modification relativement légère de la fonction de prédation peut entraîner une modification aussi marquée de la dynamique des stocks de poissons telle qu'elle est perçue, il semble que d'autres études sur les processus de la prédation dans les communautés ichtyologiques soient nécessaires avant que l'on puisse utiliser les méthodes d'évaluation multispécifiques pour acquérir une meilleure connaissance du recrutement dans les stocks de poissons.

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There have been several attempts to deal with species interactions in fish stock assessments (Mercer 1982). The North Sea model of Andersen and Ursin (1977) was one of the first large-scale attempts to describe a complicated fisheries system with several interacting species. The ecosystem model was not suitable for fish stock assessment purposes, but it prompted the development of multispecies virtual population analysis (MSVPA) methods (Sparre 1980; Pope and Knights 1982; Gislason and Helgason 1985). In these methods the natural mortality of the single-species virtual population analysis (SSVPA, e.g. Gulland 1983) is replaced by variable predation mortality and a "residual" natural mortality, which is thought to be due to, for example, disease or old age. The predation mortality is a function of predator biomass, the food "preferences" of the predators, and the amount of food available. In recent applications of the MSVPA (Gislason and Helgason 1985; ICES Ad Hoc Multispecies Assessment WG 1986) the predation mortality of a particular prey cohort has been assumed to be a slowly decreasing function of the prey cohort size (Magnus and Magnusson 1987). There does not appear, however, to be any particular justification for this type of "functional response," which resembles the type II response of Holling (1965). Studies of vertebrate prey–predator dynamics actually suggest a very different functional response, often referred to as the type III response (Holling 1965), according to which the predation mortality increases from zero to a maximum, whereafter it decreases slowly as the prey cohort size increases. The type III functional response has not received much attention in fisheries literature, although the possible effects (depensatory mortality) on the dynamics of stocks were discussed by Ricker (1954) in relation to dome-shaped stock–recruitment curves. Peterman (1977) suggested that a type III response of predators could explain some of the precipitous drops in abundance of salmonid populations.

In this paper I investigate the effects of introducing a type III functional response in the MSVPA in order to find out the

extent to which this affects the role of the predator biomass in determining recruitment and to examine the significance of uncertainty in the input data and the uniqueness of the MSVPA cohort size estimates.

Materials and Methods

Simplified MSVPA

The MSVPAs used by Gislason and Helgason (1985) and the ICES Ad Hoc Multispecies Assessment WG (1986) require large amounts of input data and have an extensive output. These features greatly complicate an understanding of the basic properties of the methods, and the sensitivity analysis. Therefore a simplified MSVPA was developed for this study by focusing on a single prey cohort and by assuming that the predator biomass remains constant. This assumption seems justified in systems such as the North Sea, where the predator biomass consists of a large number of different species with several age groups. An analysis of the results of the ICES Ad Hoc Multispecies Assessments WG (1986) also supported this assumption because the coefficient of variation of the predation mortality was less than 0.2 for a number of the stocks (Hildén 1987).

The equations that determine the dynamics of a prey cohort over the age span $a = (r,\lambda)$ were assumed to be

$$(1)\quad N_{a+1} = N_a \exp(-Z_a)$$
$$(2)\quad C_a = N_a F_a (1-\exp(-Z_a))/Z_a$$
$$(3)\quad M2_a = P_a N_a R / (N_a^2 + K_a)$$

where N_a = size of cohort, age a, C_a = catch in numbers, F_a = instantaneous fishing mortality, $Z_a = F_a + M1 + M2_a$ = instantaneous total mortality, $M1$ = instantaneous residual mortality (constant for all a), $M2_a$ = instantaneous predation mortality, P_a = parameter for the functional response of the predator biomass to changes in prey cohort size (approximate rate constant), K_a = parameter for the functional response (approximate saturation constant), and R = predator biomass.

The relationship between $M2_a$ and N_a given by equation (3) generates a sigmoid curve for the number of prey eaten by predators and a predation mortality curve with a maximum (Fig. 1). Equation (3) approximates the type III functional response because in this model the predation mortality is determined by the initial cohort size in each age group. The approximation was used because it simplied the iteration required for solving the unknowns in equations (1)–(3).

Equations (1)–(3) can be solved for F_a, N_a, and $M2_a$ if data are given for P_a, K_a, R, C_a, $M1$, and F_λ, where λ is the index for the last age group considered.

Method of Sensitivity Analysis

The role of the predator biomass in determining F_a and N_a can be studied by analysing the effects of perturbations of the predator biomass. It is, however, also desirable to consider simultaneously perturbations of the other parameters of equations (1) to (3) because the MSVPA input is subject to uncertainties (measurement errors for C_a and lack of knowledge of P_a, K_a, and $M1$). The Fourier amplitude sensitivity test (FAST) is suitable for this type of analysis (Cukier et al. 1978).

The FAST is based on a large number of simulations, each of which has a unique combination of parameter values. From the results the mean, variance, and coefficient of variation of the output variables F_a, N_a, and $M2_a$ can be obtained. The method also involves the calculation of "partial variances" which demonstrate how large a proportion of the variance in an output variable can be attributed to the perturbations of a particular parameter. In addition, approximations to linear sensitivity coefficients (Tomovic 1963) and a correlation matrix for the output variables can be obtained.

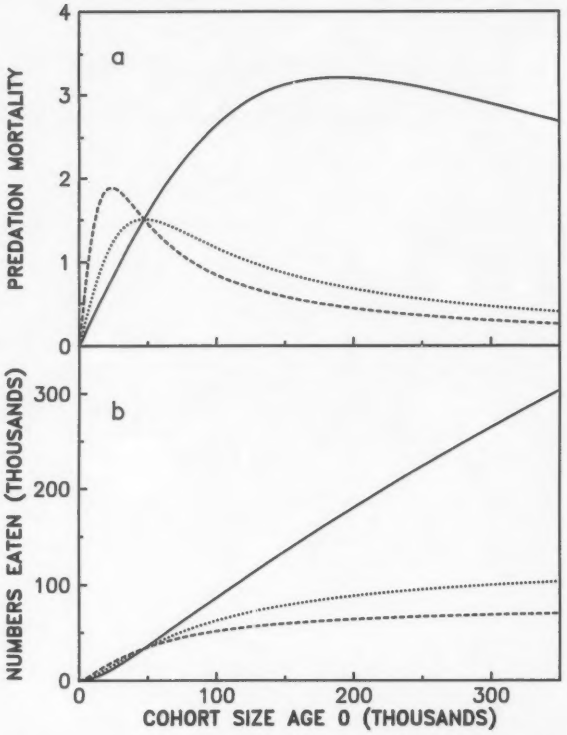

FIG. 1. Type III functional response in terms of (a) predation mortality and (b) numbers eaten by predators. The predator biomass is assumed to be constant. The different curves correspond to the nominal parameter values of the sets M2INC (continuous line), M2MAX (dotted line), and M2DEC (broken line).

Analysis Strategy

To reveal the effects of the functional response, three qualitatively different situations were analysed. In the first case, $M2_a$ was assumed to increase with the prey cohort size. In the second case, $M2_a$ changed little with the cohort size. In the third case, $M2_a$ decreased with increasing cohort size. In each case the behaviour of the solutions of equations (1)–(3) was studied by perturbing the parameter values around their nominal (most likely) values. The nominal values were generated as follows:

(1) A set of likely values was chosen for $M2_a$ in order to obtain nominal values for the parameters P_a and K_a. The nominal predation mortalities resemble closely the mean values for North Sea haddock (*Melanogrammus aeglefinus*) (ICES Ad Hoc Multispecies Assessment WG 1986).

(2) The nominal predation mortalities with given values for C_a, $M1$, and F_λ, yielded nominal solutions for F_a and N_a in an ordinary SSVPA (Table 1). The catch data resemble data for a cohort of North Sea haddock, but the values have been scaled so that $C_0 = 1000$.

TABLE 1. Nominal solutions and the nominal parameter values for the MSVPA. Note that F_6 is a parameter.

Age	Nominal solution N_a	Nominal solution F_a	Nominal solution $M2_a$	Parameter C_a	Parameter P_a M2INC	Parameter P_a M2MAX	Parameter P_a M2DEC	Parameter K_a M2INC	Parameter K_a M2MAX	Parameter K_a M2DEC	Parameter $M1$	Parameter R
0	47 416	0.0447	1.51	1000	0.8114×10^{3}	0.9546×10^{3}	0.5967×10^{2}	0.3597×10^{11}	0.2248×10^{10}	0.5621×10^{9}	0.2	1500
1	8 200	0.410	1.19	1558	0.1106×10^{3}	0.1301×10^{3}	0.8132×10^{1}	0.1076×10^{10}	0.6725×10^{8}	0.1681×10^{8}	0.2	1500
2	1 356	0.967	0.150	729	0.2305×10^{1}	0.2712	0.1695	0.2942×10^{8}	0.1839×10^{7}	0.1149×10^{6}	0.2	1500
3	363	1.27	0.050	237	0.2058	0.2422×10^{-1}	0.1514×10^{-1}	0.2111×10^{7}	0.1320×10^{6}	0.3299×10^{5}	0.2	1500
4	80	0.808	0.030	40	0.2705×10^{-1}	0.3182×10^{-2}	0.1989×10^{-2}	0.1013×10^{6}	0.6329×10^{4}	0.1582×10^{4}	0.2	1500
5	28	0.966	0.010	16	0.3193×10^{-2}	0.3756×10^{-3}	0.2348×10^{-3}	0.1267×10^{5}	0.7940×10^{3}	0.1980×10^{3}	0.2	1500
6	9	0.9764	0	5	0	0	0	0	0	0	0.2	

TABLE 2. Frequencies of the perturbations of the MSVPA parameters. Note that $P_6 = K_6 = 0$ and have therefore been assigned the same frequency.

Age	Parameter C_a	P_a	K_a	F_6	$M1$	R
0	267	1005	1405			
1	395	1101	1445			
2	487	1177	1477			
3	599	1223	1499		1543	1547
4	733	1285	1519			
5	859	1341	1529			
6	945	1367	1367	1535		

(3) The nominal solutions for $M2_a$, F_a, and N_a were fixed to three different regions on the curve relating $M2_a$ to N_a by adjusting the parameters P_a and K_a. In the following the nominal values have been marked with an asterisk and each set of runs has been given an identifier:

(a) M2INC; $M2_a$ increases with small increases in N_a:

$$K_a^* = 16(N_a^*)^2$$
$$P_a^* = M2_a^* 17\, N_a^*/R^*.$$

(b) M2MAX; $M2_a$ is at the maximum of the curve and changes relatively little with small changes in N_a:

$$K_a^* = (N_a^*)^2$$
$$P_a^* = M2_a^* 2N_a^*/R^*.$$

(c) M2DEC; $M2_a$ decreases with increases in N_a:

$$K_a^* = (N_a^*)^2/4$$
$$P_a^* = M2_a^* 5N_a^*/(4R^*).$$

The parameters were then assigned a set of frequencies incommensurate to the fourth order according to a table of Cukier et al. (1975) (Table 2). The parameters were perturbed using an even distribution with the range (nominal value/1.1, 1.1·nominal value). The number of simulations was determined using Nyquist's criterion (Cukier et al. 1978), which gave a total of 3097 simulations for each set of runs.

Results

The observed means in the sets M2MAX and M2DEC corresponded closely to the nominal solutions for all output variables (Fig. 2; Table 1). The means of the variables N_0, N_1, $M2_0$, and $M2_1$ in the set M2INC differed substantially from those of the other sets. There were, however, no significant differences in the age groups 2–6 (Fig. 2). The differences between the sets in the youngest age groups were also apparent in the coefficients of variation, which were high in M2INC (Fig. 3).

The partial variances demonstrated that most of the variance of the estimated cohort size for the youngest age groups was associated with the perturbations of the predator biomass (Table 3) whereas the perturbations of the catch data accounted for most of the variance in the age groups 2–6. The variance of the predation mortality was chiefly determined by the perturbations of the parameters P_a, K_a and R (Table 4).

For the age groups 2–6 the three sets produced nearly identical results. The only clear difference was that the catch data accounted for 5–10% of the variance in $M2_a$ in the sets M2INC and M2DEC whereas virtually no variance in $M2_a$ was associated with the catch data in M2MAX (Table 4). This difference

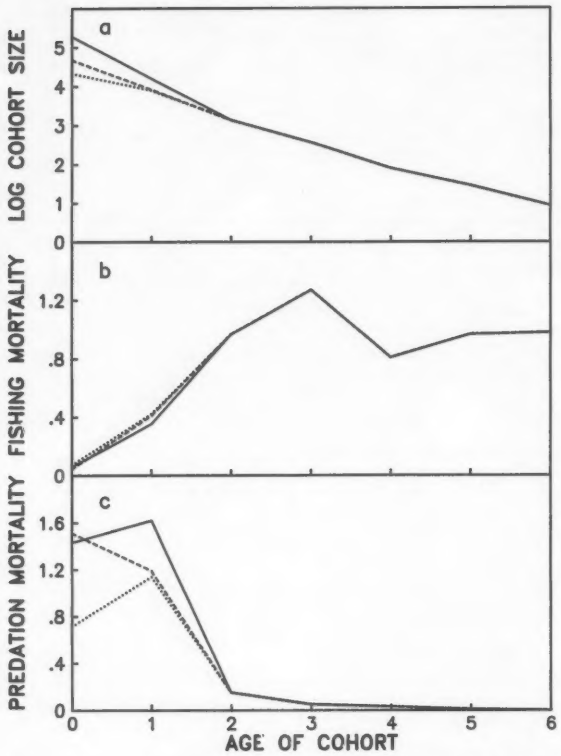

FIG. 2. Assumed nominal solutions and mean solutions in M2DEC and M2MAX (broken lines), observed nominal solutions in the set M2INC (dotted lines), and the mean of the solutions in the set M2INC (solid line). (a) Cohort size (note log scale); (b) fishing mortality; (c) predation mortality.

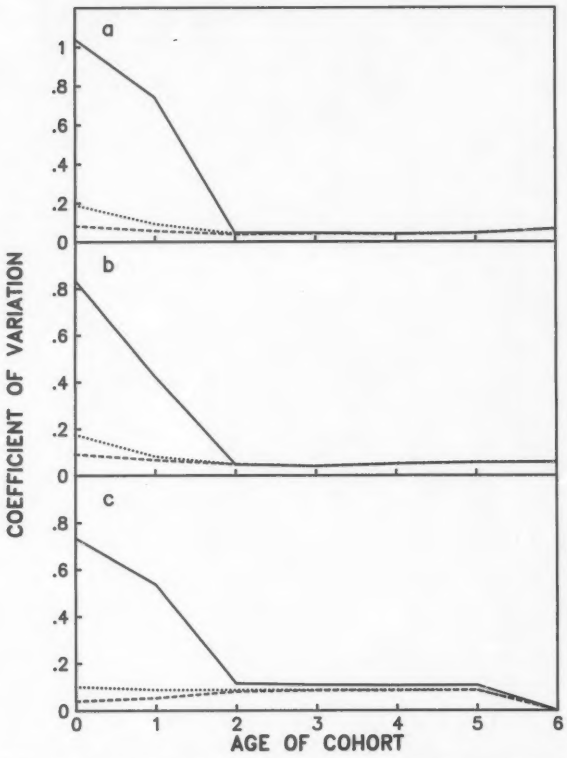

FIG. 3. Coefficients of variation for the solutions in the three different sets M2INC (solid lines), M2MAX (dotted lines), and M2DEC (broken lines). (a) Cohort size; (b) fishing mortality; (c) predation mortality.

between the sets was also apparent in the youngest age groups (Table 3).

The scaled Fourier sine coefficients, which approximate linear sensitivity coefficients, confirmed the importance of the perturbations in predator biomass as a source of variation in N_0 and N_1. They also displayed the differences between the sets in that N_0 and N_1 appeared to be one to two orders of magnitude more sensitive to perturbations in R in the set M2INC than in the sets M2MAX and M2DEC (Table 5).

The large variation in N_0 and N_1, the change in the nominal solution for N_0, and high sensitivity to perturbations in R in the set M2INC suggested that the variance of the output variables was not simply caused by fluctuations around a single nominal solution. A plot of the solutions of N_0 against N_1 confirmed this by showing three clusters of solutions. In the cluster of data points with the smallest values, the values for N_1 were around the nominal solution and the values for N_0 were around half the nominal solution. Another cluster also had values for N_1 around the nominal solution, but the values for N_0 were approximately five times the nominal solution. The largest values for the data points clustered around a solution which corresponded to three to four times the nominal solution for N_1 and nine to 10 times the nominal solution for N_0 (Fig. 4a).

Discussion

The FAST sensitivity analysis displays clearly the main properties of the simplified MSVPA. Thus the partial variances associated with parameters which determine the predation mortality are high in the youngest age groups (Table 3). In the older age groups the catch data are the most important (Table 4). These results are intuitively plausible and they also agree with a recent study of a three species system using a response surface analysis (Finn et al. 1986). One can therefore assume with some confidence that my greatly simplified MSVPA has captured the essential features of the full-scale method, although a large number of possible interactions have been excluded.

The analysis of a MSVPA with a type III functional response demonstrated that the MSVPA recruitment estimates can be sensitive to perturbations of the predator biomass. Thus, recruitment estimates from a MSVPA with a type III functional response for predators could differ substantially from estimates yielded by SSVPA in which natural mortality is assumed constant. This observation is in sharp contrast with the findings of the ICES Ad Hoc Multispecies Assessments WG (1986), which observed high correlations between SSVPA and MSVPA recruitment estimates. The main differences between the present analysis, a single-species analysis, and the analysis by the ICES Ad Hoc Multispecies Assessment WG (1986) lie in the functional response. In a single-species analysis the "natural" mortality ($M2_a + M1$) is assumed to be constant. This corre-

TABLE 3. Partial and total variances for the MSVPA output variables for the two youngest age groups under different assumptions about the change in predation mortality with cohort size. Only those parameters are included which have a partial variance >0.01 for some variable.

Run	Variable	Parameter										Sum of partial variances	Total variance
		C_0	C_1	C_2	C_3	P_0	P_1	K_0	K_1	$M1$	R		
M2INC	N_0	<0.001	0.015	0.028	0.006	0.008	0.190	0.001	0.151	0.017	0.322	0.741	0.398×10^{11}
	N_1	—	0.012	0.028	0.006	—	0.263	—	0.158	0.014	0.292	0.776	0.136×10^{9}
	F_0	0.004	0.032	0.046	0.010	0.004	0.191	0.003	0.178	0.029	0.321	0.821	0.245×10^{-2}
	F_1	—	0.001	0.054	0.012	—	0.250	—	0.189	0.023	0.294	0.827	0.223×10^{-1}
	$M2_0$	<0.001	0.023	0.031	0.006	0.014	0.115	0.008	0.132	0.019	0.285	0.637	1.11
	$M2_1$	—	0.013	0.024	0.005	—	0.264	—	0.188	0.012	0.293	0.801	0.757
M2MAX	N_0	<0.001	0.010	0.016	0.003	0.203	0.079	0.053	0.020	0.025	0.572	0.981	0.795×10^{8}
	N_1	—	0.041	0.065	0.014	—	0.342	—	0.085	0.035	0.398	0.983	0.567×10^{6}
	F_0	0.099	0.014	0.022	0.005	0.114	0.110	0.034	0.029	0.026	0.480	0.934	0.661×10^{-4}
	F_1	—	0.306	0.121	0.025	—	0.190	—	0.051	0.036	0.248	0.982	0.111×10^{-2}
	$M2_0$	<0.001	<0.001	<0.001	<0.001	0.383	0.002	0.107	0.001	0.001	0.436	0.930	0.222×10^{-1}
	$M2_1$	—	<0.001	<0.001	<0.001	—	0.436	—	0.111	<0.001	0.437	0.984	0.107×10^{-1}
M2DEC	N_0	<0.001	0.004	0.007	0.001	0.297	0.045	0.012	0.001	0.020	0.596	0.983	0.153×10^{8}
	N_1	—	0.036	0.055	0.011	—	0.387	—	0.011	0.033	0.447	0.983	0.212×10^{6}
	F_0	0.380	0.007	0.010	0.002	0.106	0.072	0.005	0.002	0.015	0.381	0.980	0.170×10^{-4}
	F_1	—	0.544	0.101	0.021	—	0.113	—	0.008	0.028	0.162	0.981	0.714×10^{-3}
	$M2_0$	<0.001	0.007	0.010	0.002	0.603	0.071	0.025	0.002	0.024	0.238	0.982	0.355×10^{-2}
	$M2_1$	—	0.020	0.025	0.005	—	0.474	—	0.016	0.012	0.430	0.983	0.420×10^{-2}

sponds to a type I functional relationship and the numbers killed increase linearly with prey cohort size. The ICES Ad Hoc Multispecies Assessment WG (1986) has effectively assumed a type II functional response in which the predation mortality increases with decreasing prey cohort size.

The analysis of the simplified MSVPA demonstrated that assumptions regarding the predation process are critical. In terms of a "complete" MSVPA, such as that of Gislason and Helgason (1985), this means that the "suitability" coefficients and their response to changes in the abundance of the prey cohort have a major impact on the results of the analysis.

The present demonstration of the importance of a type III functional response is in line with earlier theoretical studies of predator–prey systems, in which type III responses have been shown to create multiple stable population levels and limit cycles (reviews by Svirezhev and Logofet 1983; Beddington 1984).

In the MSVPA the reason for the peculiar results in the set M2INC is revealed by a plot of cohort size at age 0 against cohort size at age 1 for the three sets of nominal parameter values. In the set M2INC the curve bends backwards, and for a range of cohort sizes at age 1 there is thus not a unique correspondence with cohort sizes at age 0 (Fig. 4b). This problem does not arise in a single-species analysis in which the predation mortality is assumed to be constant, i.e. with a type I functional response. Because of the monotonic change in predation mortality in a type II response, the problem was also not encountered in the North Sea MSVPA application by the ICES Ad Hoc Multispecies Assessment WG (1986).

For any set of fixed parameter values P_a, F_a, R, and $M1$, there is a range of cohort sizes N_a and values of the parameter K_a for which the curve relating N_{a+1} to N_a will bend backwards in the type III response. This can be investigated by determining sign changes in the derivative $d(N_{a+1})/d(N_a)$ (Fig. 5). The different results of the three sets of runs are now easy to explain. The nominal values N_0 and K_0 of the set M2INC were in the region of negative derivatives, and perturbations of the parameter values caused the solutions to drift from this region. In the sets M2MAX and M2DEC the nominal values were well removed from the critical region, and substantial parameter perturbations would have been required to create the effects observed in M2INC.

In the MSVPA the predation mortality parameters are age dependent (equations (1)–(3)). Thus the role of the predators in determining changes in the cohort size changes with the age of the cohort (Fig. 6). Figure 6 also explains the positions of the three clusters in Fig. 4a. The first cluster corresponds to solutions which have been obtained from the solution of low values in N_1 and low values in N_0. The second cluster has arisen from intermediate solutions for N_1 and high levels of N_0 (intersection of vertical line and the N_0–N_1 curve at approximately $5N_0$). Finally, the cluster of high values has been obtained from the solutions which have drifted to high values as early as age group 1 (intersection of vertical line by the N_1–N_2 curve at approximately $4N_1$). These correspond to a solutions clustered around $9N_0$. Note that the solution on the backward bending part of the curve relating two adjacent cohort sizes (Fig. 6) corresponds to unstable equilibria in a predator–prey system (Peterman 1977). This explains why no solutions corresponding to the nominal solution for N_0 were found in the sample (Fig. 4).

The loss of uniqueness of the cohort size estimates complicates somewhat the interpretation of the partial sensitivities and the sensitivity coefficients of the FAST method. Because it is based on a Fourier analysis, an inherent assumption is that the model represents a continuous function. This is not the case when the functional response creates a bifurcation of the solutions. The high sine coefficients for the predator biomass in the set M2INC (Table 5) are thus only partly due to the perturbations of the predator biomass per se. Most of the observed variance is structural and arises because of the nonuniqueness of the solutions. Different results can therefore be obtained depending on the algorithm used for calculating the cohort sizes-at-age. The algorithm used in this study did not, however, seem to discriminate systematically against any of the solutions, and

TABLE 4. Partial and total variances of the MSVPA output variables for age groups 2–6 in the set M2MAX. Only partial variances >0.005 are included. Note that F_6 is a parameter and that $M2_6 = 0$.

Variable	Parameter												Sum of partial variances	Total variance
	C_2	C_3	C_4	C_5	C_6	P_2	P_3	P_4	P_5	K_2	K_3	K_4	K_5	F_6	$M1$	R		
N_2	0.711	0.147	0.007			0.017									0.072	0.022	0.983	0.314×10^4
N_3		0.868	0.044	0.011											0.051		0.982	0.251×10^3
N_4			0.639	0.163	0.065									0.024	0.092		0.984	9.80
N_5				0.610	0.244									0.090	0.041		0.985	1.61
N_6					0.715									0.264	0.005		0.984	0.349
F_2	0.592	0.301	0.015			0.005									0.052	0.009	0.982	0.205×10^{-2}
F_3		0.642	0.193	0.049	0.020									0.007	0.071		0.985	0.258×10^{-2}
F_4			0.593	0.223	0.089									0.033	0.045		0.983	0.167×10^{-2}
F_5				0.409	0.410									0.150	0.016		0.985	0.302×10^{-2}
$M2_2$						0.436				0.111						0.436	0.983	0.170×10^{-3}
$M2_3$							0.437				0.110					0.437	0.984	0.188×10^{-4}
$M2_4$								0.437				0.110				0.436	0.983	0.678×10^{-5}
$M2_5$									0.437					0.110		0.437	0.984	0.751×10^{-6}

TABLE 5. Scaled sine Fourier coefficients for predator biomass (R) in the three sets with and without perturbations of the parameters P_a and K_a (equation (3)). The coefficients approximate the change in the variables N_0 and N_1 brought about by a perturbation of one unit of the predator biomass.

	Variable			
	N_0		N_1	
Run	Perturbation of all parameters	No perturbation of P_a and K_a (a = [0,6])	Perturbation of all parameters	No perturbation of P_a and K_a (a = [0,6])
M2INC	92.6	131	13.8	18.2
M2MAX	2.48	2.49	1.01	1.02
M2DEC	1.11	1.09	0.651	0.647

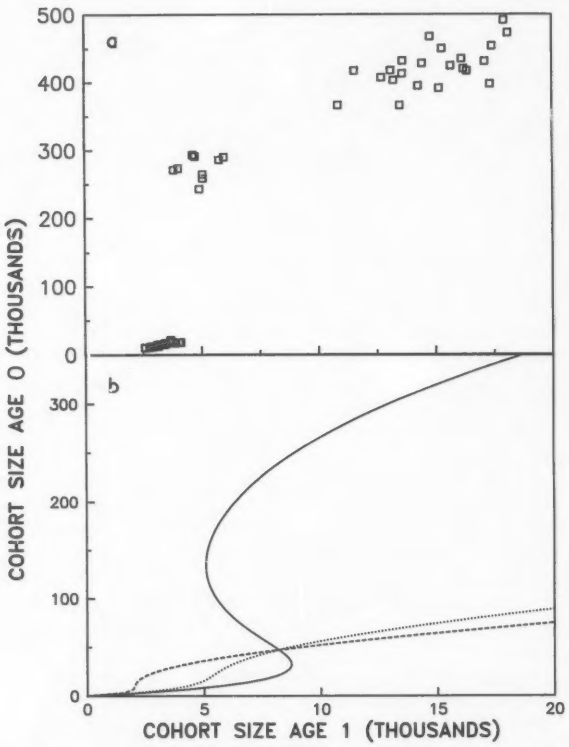

FIG. 4. (a) Solutions for N_0 plotted against the solutions for N_1 from a sample of 61 solutions of the set M2INC (approximately every fiftieth solution). (b) Plot of N_0 against N_1 for the three different sets of nominal parameter values (Table 1). M2INC = solid line; M2MAX = dotted line; M2DEC = broken line. The point of intersection of the lines gives the nominal solution.

the runs displayed the full range of possible solutions (Fig. 4). The set M2INC therefore displays a great potential for predator-induced recruitment variability.

The problems with multiple solutions occur only in the youngest age groups in species such as the haddock, whose predation mortality decreases rapidly with age (Gislason and Helgason 1985). Consequently the differences between SSVPA and MSVPA estimates should be negligible in the older age groups, and this assumption is supported by the similarity of

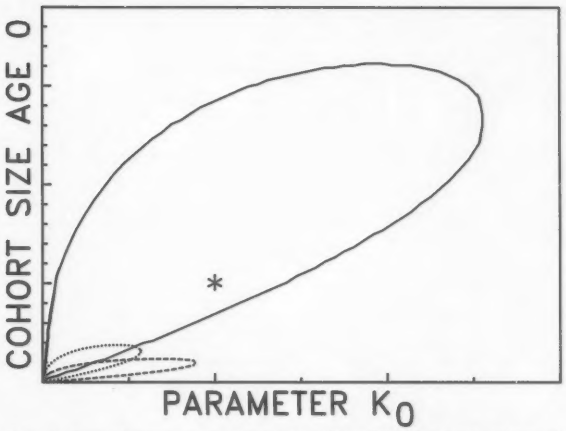

FIG. 5. Sign changes in the derivative $d(N_1)/d(N_0)$ for fixed values of P_0, R, F_0, and $M1$. The derivative becomes negative in the area delimited by the curve. M2INC = solid line; M2MAX = dotted line; M2DEC = broken line. The asterisk marks the position of the nominal values in the three sets of simulations.

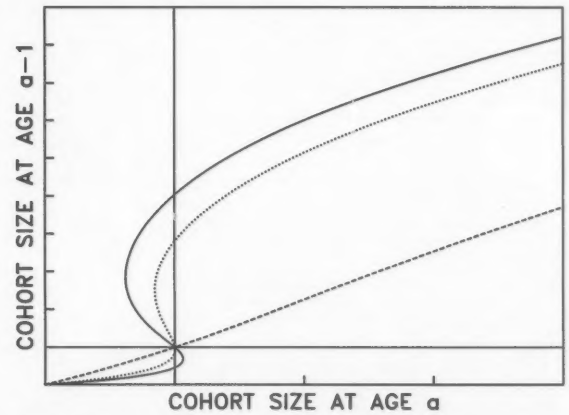

FIG. 6. Age dependency of the plot of N_a against N_{a-1} in the set M2INC. Although the functional response is the same for all age groups, the retroversion occurs only in the two youngest age groups. Age 1–0 = solid line; age 2–1 = dotted line; age 3–2 = broken line. All curves have been scaled to the nominal values. The intersection of the two straight lines give the assumed nominal solutions.

the set of runs in the age groups 2–6 (Fig. 2 and 3). The situation may be very different for species such as the sprat (*Clupea sprattus*) which experiences a high predation mortality throughout its life span. For such species the perceived dynamics of the population may change radically depending on the basic method of analysis (SSVPA or MSVPA) and the choice of functional response in the MSVPA.

The observation of possible multiple solutions in a MSVPA suggests that the stability of the method for catch data analysis depends on the assumptions of the predation process. A recent analysis of the uniqueness and existence of the solutions of a MSVPA with a type II functional response demonstrated that uniqueness problems will not arise until the predation mortalities increase above 2 (Magnus and Magnusson 1987). The present study has shown that this limit is too optimistic for a MSVPA with a type III functional response.

If a type III functional response is a valid description of the predation process in fish communities, the results above will have far-reaching consequences. There is theoretical support for this type of predator response, and Peterman (1977) has reviewed a number of feeding strategies of fish predators which could lead to a type III response. Note, however, that not only the response curve but also its parameters are significant. Thus, if the environmentally induced year class fluctuations are at a level where the predators are incapable of increasing the predation mortality, a constant predator biomass cannot change the relative size of a year class. This situation is illustrated in the sets M2MAX and M2DEC and corresponds to the current paradigm of the ICES Ad Hoc Multispecies Assessment WG (1986). The set M2INC demonstrates that if predators respond by significantly increasing the predation on strong year classes, then even a constant predator biomass may change the relative strengths of year classes.

The central question that emerges is whether predators can alter the year class strengths in fish stocks simply by responding to changes in prey abundance. This question has not been dealt with in the recent practical applications of MSVPAs to North Sea stocks, which partly explains why these MSVPAs have not been able to improve the understanding of recruitment variability. A number of studies reviewed by Peterman (1977) have shown that predators can control salmonid recruitment and that depensatory juvenile mortality can occur, but similar empirical evidence is scarce for marine fish communities. In recent enclosure experiments, however, Øiestad (1985) has demonstrated the significance of predators for larval survival. Additional indications of predator control of year class strength may be obtained by investigating changes in relative abundance at different life history stages, since some of these changes could be caused by predators feeding according to a type III functional response (Svirezhev and Logofet 1983).

A comparison of the ranking of year class strengths based on the International Young Fish Survey (IYFS) and VPA recruitment estimates in the North Sea demonstrates that changes in relative abundance do indeed occur (Table 6). Some of the changes may of course be due to sampling errors. For example the VPA estimates for cod year class strength correlate better with the English groundfish survey index than with the IYFS index (K. Stokes, Fisheries Laboratory, Lowestoft, U.K., pers. comm.). Apart from the cod, however, the species that show the most frequent change in relative abundance are those that, according to the estimates of Gislason and Helgason (1985), experience the highest predation mortality rates. It is possible that some of the changes in relative abundance could be explained by a MSVPA which takes into account a type III functional response of predators to changes in prey cohort size. This could also help to improve the understanding of the recruitment process, providing an explanation of some of the fluctuations that have previously been related to unspecified environmental changes (wind, temperature, etc.). Such an analysis will require detailed information on the food and feeding strategies of fish predators.

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TABLE 6. Differences in year class strength ranking based on the International Young Fish Survey Index (IYFS) and the VPA results for 1-yr-old fish in the North Sea, and the correlation between the index and the abundance estimate (data from the ICES Ad Hoc Multispecies Assessment WG 1986).

	VPA ranking					
IYFS ranking	Cod (*Gadus morhua*) (r = 0.416)	Haddock (r = 0.944)	Whiting (*Merlangus merlangus*) (r = 0.908)	Herring (*Clupea harengus*) (r = 0.859)	Norway pout (*Trisopterus esmarkii*) (r = 0.840)	Sprat (r = 0.066)
1	4	3	1	3	1	8
2	2	1	2	2	2	5
3	8	4	3	1	5	3
4	1	2	4	5	3	6
5	7	6	6	7	4	1
6	6	7	9	6	7	4
7	3	5	8	4	6	2
8	10	9	5	8		7
9	9	8	7	9		
10	5	10	10			

number of points. Veijo Kaitala examined the manuscript critically and pointed out the problems which arise in using the FAST method when the solutions are nonunique. Anna Damström checked the English and Lisa Rosenqvist typed the manuscript. The study was supported financially by the University of Helsinki (Herman Rosenberg Grant) and the Finnish Game and Fisheries Research Institute. Permission by ICES to cite the report of the Ad Hoc Multispecies Assessement Working Group is also gratefully acknowledged.

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The Mercury Problem in Recently Formed Reservoirs of Northern Manitoba (Canada): Effects of Impoundment and Other Factors on the Production of Methyl Mercury by Microorganisms in Sediments

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Creation of hydroelectric reservoirs by enlargement of riverine lakes and flooding of adjacent forested land along the Churchill River diversion route has led to a marked rise in rates of methyl mercury production by microorganisms in sediments. This phenomenon has resulted primarily from stimulation of microbial activity by organic matter in submerged land areas and is due both to utilization of organic nutrients by methylators and to concomitant oxygen depletion. Release of mercury from scattered low-level sources in this organic matter is a secondary contributing factor. Compared with submerged terrestrial organics, organic matter from aquatic biota has only been of minor importance in promoting methyl mercury production. In some regions, clay and silt eroded from shoreline deposits have inhibited methyl mercury production appreciably. The aerobic conditions prevailing in well-flushed main-stream regions tend to increase the "availability" of sediment-bound inorganic mercury for methylation while decreasing the rate at which microbes are able to methylate the mercury; under less aerobic conditions in quiet backwater regions, the reverse is true.

La création de réservoirs hydroélectriques par l'agrandissement de lacs situés en bordure de rivières et l'inondation des forêts avoisinantes dans le cadre du projet de dérivation de la rivière Churchill a entraîné une augmentation marquée des taux de production de mercure-méthyle par les micro-organismes présents dans les sédiments. Ce phénomène est en grande partie le résultat de la stimulation de l'activité microbienne par les matières organiques présentes dans les terres immergées; il est causé par l'utilisation des bioéléments organiques par les méthylateurs et l'appauvrissement incident en oxygène. La libération du mercure de sources dispersées à faible teneur au sein de la matière organique produite par le biote aquatique ne joue qu'un rôle mineur dans la stimulation de la production de mercure-méthyle par rapport aux matières organiques terrestres immergées. Dans certaines régions, l'argile et le limon provenant de l'érosion des rives ont nettement inhibé la production de mercure-méthyle. Les conditions aérobiques qui règnent dans les parties du cours principal bien drainées ont tendance à accroître la « disponibilité » pour la méthylation du mercure inorganique lié aux sédiments tout en ayant tendance à abaisser le taux de méthylation du mercure par les microbes; en présence de conditions moins aérobiques dans les secteurs d'eau dormante, la situation contraire s'applique.

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The creation of reservoirs by the impoundment of river water and flooding of adjacent land commonly causes an appreciable increase in the mercury (Hg) content of fish inhabiting the water (Gebhards et al. 1971; Potter et al. 1975; Abernathy and Cumbie 1977; Bruce and Spencer 1979; Cox et al. 1979; Bodaly and Hecky 1979; Meister et al. 1979; McGregor 1980; Waite et al. 1980; Bodaly et al. 1984a; Jackson 1984b, 1987). Because this phenomenon has occurred repeatedly in newly formed reservoirs located in widely separated, environmentally diverse geographical regions where no anthropogenic or natural point sources of Hg contamination are known to exist, and has not been observed in nearby bodies of water unaffected by flooding, it is believed to be a general, if not universal, effect of impoundment. The literature cited above suggests that the elevated rates of Hg accumulation by fish in new reservoirs are due to either or both of two processes: (1) release of Hg into the aquatic ecosystem from scattered low-level sources in flooded land and (2) enhancement of Hg mobility and bioavailability, owing in particular to accelerated microbial transformation of "inorganic" (nonmethyl) Hg into methyl mercury (CH_3Hg^+). CH_3Hg^+, a highly toxic lipophilic species, accumulates in fish tissues preferentially with respect to other forms of Hg (Huckabee et al. 1979).

A major cause of the Hg problem in new reservoirs is thought to be stimulation of microbial methylation of Hg in sediments (along with heterotrophic microbial growth and activity in general) by nutrients — especially labile organic substances — introduced into the aquatic environment from recently submerged soil and vegetation (Bodaly et al. 1984a; Hecky et al. 1987; Jackson 1984b, 1987). However, detailed knowledge of

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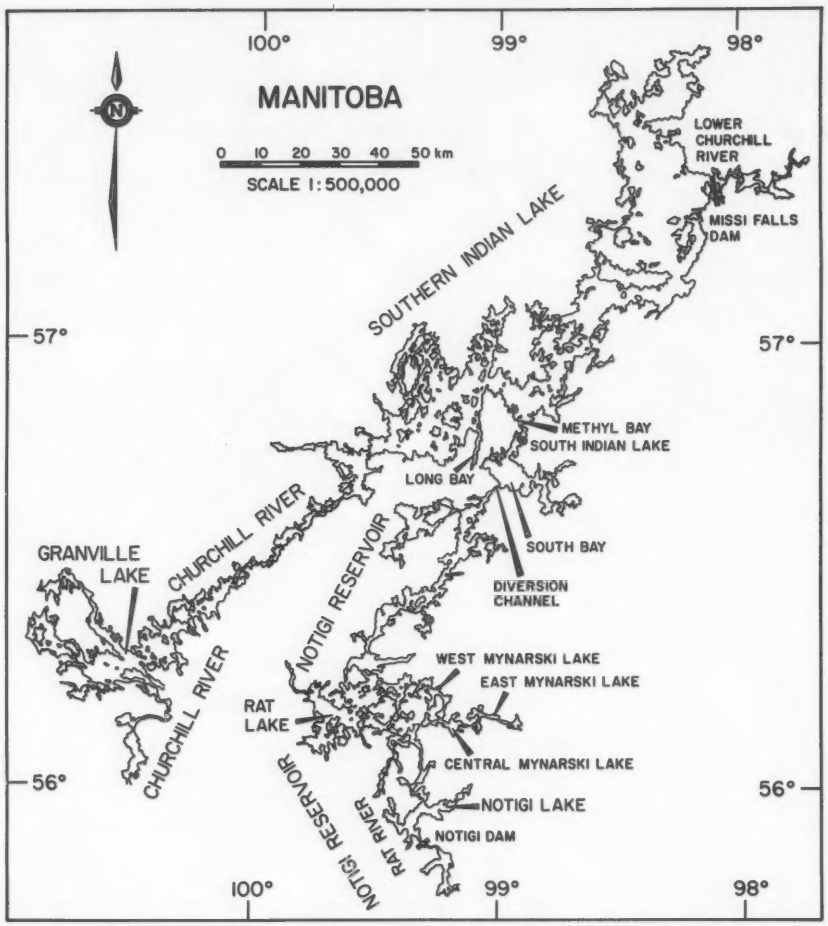

FIG. 1. Field area. *Note*: "South Indian Lake" is a village on the shore of Southern Indian Lake.

the mechanisms involved has been lacking. Impoundment gives rise to a complex of interrelated physicochemical and biological changes, any of which could have direct or indirect effects on the biogeochemical pathways of Hg. Some of these effects may tend to cancel each other. For instance, Hg methylation may be counteracted by decomposition ("demethylation") of CH_3Hg^+ (Fagerström and Jernelöv 1972; Jernelöv 1972; Shariat et al. 1979; Jackson 1987), and the decaying organic matter which stimulates CH_3Hg^+ production may also inhibit the process through the formation of sulifides, which bind Hg strongly, thereby lowering its availability for methylation (Jernelöv 1972; Jackson 1987). Moreover, Hg concentrations in biota could, theoretically, be reduced as a result of "biomass dilution" (Jernelöv et al. 1975; W. F. Warwick, National Hydrology Research Institute, Saskatoon, Sask. S7N 3H5, pers. comm.) following the temporary increase in biological production that generally occurs soon after impoundment (Jackson and Hecky 1980). Nevertheless, the *net* effect of impoundment generally appears to be a rise in the Hg content of fish.

The present paper reports results of research on the causes of a Hg problem which resulted from a hydroelectric project (the Churchill River diversion) in northern Manitoba, Canada. Impoundment and rerouting of river water caused enlargement of several riverine lakes, and this led to marked environmental and biological changes within the affected lakes (Hecky and Harper 1974; Hecky et al. 1979; Guilbault et al. 1979; Jackson and Hecky 1980; Can. J. Fish. Aquat. Sci. 41(4): 548–732 (1984, special issue devoted to the Churchill River diversion); Jackson 1984b, 1987). Harmful effects on local fishing interests had been predicted (Dickson 1972), and indeed, environmental changes such as increased turbidity due to accelerated shoreline erosion have caused a drastic decline in the quantity and quality of the commercial whitefish catch in the region (Bodaly et al. 1984b). Among the effects of the Churchill River diversion has been a sharp rise in the Hg content of fish in the new reservoirs, starting within 2 yr following the onset of impoundment and shoreline flooding (Bodaly and Hecky 1979; McGregor 1980; Bodaly et al. 1984a), while fish in nearby lakes unaffected by inundation have remained at background levels. In the reservoirs, the mean Hg concentrations of pike and walleye muscle tissue have risen above the 0.5 μg/g limit imposed by the Federal Food and Drug Directorate on fish marketed in Canada.

This report deals with effects of environmental factors on CH_3Hg^+ levels and CH_3Hg^+ production rates in sediments of

TABLE 1. Information on the sampling sites. — = no sample.

Sampling site	Main stream (MS) or backwater (BW)	Rise in water level after flooding (m)	Sampled (+) or not sampled (0) in years indicated		Depth of water at sampling site (m)			Water strongly to moderately stratified (SS), weakly stratified (WS), or unstratified (U)		
			1981	1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983
Granville Lake	MS	0	+	+	21	—	11.5	U	—	U
East Mynarski Lake	BW	0	0	+	—	—	14.5	—	—	SS
Southern Indian Lake										
Long Bay (shallow cove)	BW	3	+	+	4.5	4	4.5	U	U	U
Long Bay (main basin)	BW	3	+	+	6.5	—	6.5	WS	—	U
South Bay	MS	3	+	+	5.5	6	4.7	U	U	WS
Methyl Bay (flooded forest)	BW	3	0	+	—	—	2	—	—	U
Methyl Bay (offshore)	BW	3	0	+	—	—	4.75	—	—	WS
Notigi Reservoir										
Rat Lake	MS	10.1	+	+	16	15	10.5	WS	SS[a]	SS[a]
West Mynarski Lake	BW	9.0	0	+	—	—	9.25	—	—	SS
Central Mynarski Lake	BW	6.9	+	+	16	14.9	10.5	WS	SS	SS
Notigi Lake (west basin)	MS	15.2	+	+	17.5	15	19	U	SS	SS
Notigi Lake (east basin)	BW	15.2	+	+	26	24	17.5	SS	SS	SS[a]

[a]Epilimnion and thermocline present, but no hypolimnion.

different lakes in northern Manitoba, with particular attention to effects of environmental changes due to postimpoundment flooding. The primary objective of the study was to test the hypothesis that the Hg problem resulted from stimulation of microbial methylating activity by nutrients or available inorganic Hg, or both, released from recently inundated land. The method adopted was to combine the analysis of sediment and water samples collected in the field with the performance of laboratory experiments designed to test the Hg methylating capabilities of the sediments under controlled conditions. Related research on the bioavailability and bioaccumulation of Hg in the lakes will be published separately.

Field Area

The field area (Fig. 1) is located between latitudes 55 and 57°N in a region of low relief, moist sub-Arctic continental climate, podzolic soil, and Boreal forest dominated by black spruce (*Picea mariana*). The bedrock consists of Precambrian igneous and metamorphic rocks largely overlain by Pleistocene glacial sediments, including much varved glacio-lacustrine silty clay. Owing to the buffering action of carbonates in the Pleistocene deposits, surface waters of the region commonly have neutral to weakly alkaline pH values despite an abundance of humic matter derived from local soil and muskeg (Jackson and Hecky 1980).

Reservoir creation occurred during 1974–76, when water of the Churchill River, which flows eastward through Granville Lake and thence into Southern Indian Lake, was impounded at the natural outflow of Southern Indian Lake (Missi Rapids) and rerouted southward out of South Bay and into the Rat River system by way of an artificial diversion channel (Fig. 1). As a result, the water level of Southern Indian Lake has risen by 3 m, and the lake's mean surface area has increased by 21%, while the complex of interconnected lakes called "Notigi Reservoir," which includes Rat Lake, Notigi Lake, West Mynarski Lake, and Central Mynarski Lake, has risen by 6.9–15.2 m (the exact increase depending on the individual lake), and its mean surface area has increased by 282% (Bodaly et al. 1984a). Owing to the uniformly low relief of the region, the rise in water level is a reasonable estimate of the relative increase in surface area.

The lakes investigated (Fig. 1) were Granville Lake and Southern Indian Lake of the Churchill River system, Rat Lake and Notigi Lake of the Rat River system, and the three Mynarski lakes (East, Central, and West), which form a continuous waterway flowing into Rat Lake. The east and west basins of Notigi Lake were sampled separately, and samples were taken from different regions of Southern Indian Lake, specifically South Bay, Long Bay (now officially called Wupaw Bay), and Methyl Bay (an informal name coined by scientists working in the area). There were two sampling sites in Long Bay: the main basin and a small shallow cove. In Methyl Bay, samples were collected from both a "Flooded Forest" zone of recently submerged land and from an "offshore" zone of preimpoundment lake bottom. The Flooded Forest site of Methyl Bay was the only sampling locality situated directly over a land surface inundated because of the Churchill River diversion. All other reservoir sites were located sufficiently far offshore to have been lacustrine even prior to impoundment.

Granville Lake and East Mynarski Lake have not been affected by the Churchill River diversion at all and therefore represent preimpoundment background conditions. Granville Lake is designated a "control" region for purposes of comparison with Southern Indian Lake and Notigi Reservoir lakes. However, East Mynarski Lake (along with West Mynarski Lake) is in a class by itself, being distinguished by anomalously high rates of biological production owing to favourable local conditions (see Table 8 (below) and Bodaly et al. 1987).

As might be expected, environmental effects of flooding tend to increase in the order Granville < Southern Indian Lake < Notigi Reservoir lakes (Bodaly et al. 1984a). Nevertheless,

TABLE 2. List of the solvents used for extraction of different forms of Hg, Fe, and Mn from sediments, and metal fractions presumably extracted by these solvents, together with references to literature describing the extraction procedures.

Solvent or solvent mixture	Abbreviated name used to designate the solvent or solvent mixture in the text	Metal fractions assumed to have been extracted by the solvents	Reference(s)
0.5 *M* $CaCl_2$	$CaCl_2$	Exchangeable, weakly adsorbed (bioavailable?) species	McLaren and Crawford 1973
2.5% acetic acid	HAc	Species adsorbed to clays, oxides, and organic matter; bioavailable species (?)	McLaren and Crawford 1973
0.005 *M* diethylenetriaminepentaacetic acid (DTPA) + 0.01 *M* $CaCl_2$ + 0.1 *M* triethanolamine (TEA)	DTPA	Adsorbed and complexed species; metals occluded in labile solid phases (e.g. hydrated oxides); bioavailable species (?)	Lindsay and Norvell 1978
0.1 *M* $NH_2OH{\cdot}HCl$ + 0.01 *M* HNO_3	$NH_2OH{\cdot}HCl$	"Amorphous" readily reduced hydrated Fe and Mn oxides (mainly MnOOH)	Chao 1972; Gupta and Chen 1975
0.27 *M* citrate + 0.11 *M* dithionite	Citrate/dithionite	Relatively refractory, relatively crystalline hydrated Fe and Mn oxides (mainly FeOOH)	McKeague and Day 1966; Holmgren 1967; Gupta and Chen 1975
0.1 *M* NaOH	NaOH	Metals complexed and adsorbed by humic matter and associated non-humic organics and peptized FeOOH	Schnitzer and Khan 1972

owing to local environmental peculiarities predating the Churchill River diversion, regional variations in the physicochemical and biological characteristics of the lakes are not necessarily due simply to variations in the extent of flooding. Among these complicating circumstances are the following: (1) Some sampling sites are in "main-stream" (or main-stem) regions that are vigorously flushed by river water, while others are in lower-energy "backwater" regions, where flushing is slower and the influence of local drainage is more pronounced, as shown by higher concentrations of terrigenous substances such as allochthonous humic matter (Jackson and Hecky 1980). (2) Unlike the other sampling regions, South Bay is bordered by Pleistocene silty clay deposits, which are undergoing rapid erosion along the shoreline, causing an anomalously high degree of turbidity and creating a gradational decrease in suspended detrital load in the order South Bay > Rat Lake > Notigi Lake (Hecky and McCullough 1984). Whereas South Bay is richer in silt and clay than Notigi Reservoir, the Notigi Reservoir lakes (especially the backwater zones) are richer in organic substances such as allochthonous humic matter. Thus, it will be necessary to examine the possibility that eroded silty clay as well as extent of flooding has a bearing on the Hg problem. (3) Granville Lake and the west basin of Notigi Lake have certain biological and geochemical anomalies in common, such as high primary productivity (R. E. Hecky, Freshwater Institute, Winnipeg, Man., pers. comm.) and manganese nodules, which set them apart from the other sampling regions. (4) East Mynarski Lake and, to a lesser extent, West Mynarski Lake have unusually high levels of primary productivity (see Table 8 (below) and Bodaly et al. 1987).

Materials and Methods

Sample Collection

Samples were collected at the field sites (Table 1; Fig. 1) during 23–25 June 1981 (approximately at the time of the spring flood) and 11–13 August 1981 and also 12–15 August 1983 and 5–6 June 1984. In 1983, samples were taken from all of the 1981 sites and from several additional localities. Only a few selected sites were sampled in 1984.

Water samples were collected just below the surface and 1 m or less above the bottom with a van Dorn bottle or with plastic jugs lowered by hand. Fine-grained bottom sediments (approximately the top 10–15 cm) were collected with an Ekman dredge. Land plants, including sphagnum moss used in methylation experiments, were sampled at three sites in the woods near the shore of Southern Indian Lake, and at one of these sites the "A" and "B" horizons and leaf litter of the soil were sampled. A varved Pleistocene clay–silt deposit on the shore of Southern Indian Lake (South Bay) was also sampled.

Sample Preservation, Preparation, and Storage

Sediment samples were kept temporarily in a cool, dark storage space at a field camp and were transported back to the laboratory (at the Freshwater Institute) within a few days of collection, whereupon they were stored at 4°C. Sediment subsamples to be analyzed for total Hg, solvent-extractable "available inorganic Hg" fractions, and sulfide were kept in their native state at 4°C, while subsamples reserved for CH_3Hg^+ analysis were frozen. Other subsamples were oven-dried at 105°C or freeze-dried. Sediments to be used for laboratory experiments were stored at 4°C in plastic bags from which air had been excluded.

Water samples were subdivided into whole water, filtered water, and centrifuge supernates. Filtration was done with 0.45-μm Millipore filters precleaned with dilute HCl and preweighed. Centrifugation was done in two stages: (1) immediately after collection, the water was subjected to continuous-flow centrifugation at 500 mL/min and 10 000 rpm (≃ 9220 × *g*) at the field camp; (2) several days later, back in the laboratory, the spun-down particles were centrifuged again at 20 000 rpm (≃ 36 900 × *g*) for 20 min. Subsamples of water

TABLE 3. Description of sediment samples collected in 1981 and 1983, together with E_h and pH values for 1983 sediments. — = no sample.

Sampling site	Description of sediments 1981	Description of sediments 1983	E_h	pH
Granville Lake	Mottled grey and brown mud; Mn nodules present	Mud with orange–brown surface floc	+13	6.83
East Mynarski Lake		Grey mud with black mottling; slight sulfide odour	−102	7.08
Southern Indian Lake				
Long Bay (shallow cove)	Brown–grey mud; no sulfide odour	Brown–grey mud with 0.5-cm oxidized layer	−30	6.80
Long Bay (main basin)	Grey and brownish mud; no sulfide odour.	Grey–brown mud with 0.5- to 1-cm oxidized layer	−20	6.82
South Bay	Brownish–grey mud	Grey mud with 2-cm reddish brown oxidized layer	+30	7.00
Methyl Bay (flooded forest)	—	Grey mud with much plant detritus; slight sulfide odour	+20	6.70
Methyl Bay (offshore)	—	Grey-brown mud with 1.5-cm orange–brown oxidized layer	+50	6.60
Notigi Reservoir				
Rat Lake	Mottled grey–brown mud; no sulfide odour	Grey mud	−60	6.95
West Mynarski Lake	—	Grey mud	−95	7.08
Central Mynarski Lake	Mottled brown–grey mud with black and ochre lenses; sulfide odour	Grey mud	−125	7.25
Notigi Lake (west basin)	Mottled grey and brown mud; sulfide odour in August; Mn nodules present	Grey mud with 2-cm oxidized layer	−120	7.15
Notigi Lake (east basin)	Grey and brown mud with oxidized layer a few millimetres thick; no sulfide odour	Grey mud with black plant detritus; slight sulfide odour	−94	6.84

TABLE 4. Grain size frequencies of sediments and percentage loss of weight on digestion of the sediments with H_2O_2. Size classes are defined by the following ranges of particle diameters: clay, <4.0 μm; silt, 4.0–62.5 μm; sand, 62.5–2000 μm.

Sampling site	% clay June 1981	% clay Aug. 1981	% clay Aug. 1983	% silt June 1981	% silt Aug. 1981	% silt Aug. 1983	% sand June 1981	% sand Aug. 1981	% sand Aug. 1983	% loss on digestion with H_2O_2 June 1981	% loss on digestion with H_2O_2 Aug. 1981
Granville Lake	28.45	—	77.0	29.04	—	23.0	42.50	—	0.0	8.57	—
East Mynarski Lake	—	—	72.0	—	—	28.0	—	—	0.0	—	—
Southern Indian Lake											
Long Bay (shallow cove)	83.13	70.87	88.0	16.24	20.38	12.0	0.63	8.75	0.0	5.88	7.29
Long Bay (main basin)	76.41	—	80.0	23.47	—	20.0	0.12	—	0.0	2.92	—
South Bay	49.23	46.46	63.0	50.41	52.95	37.0	0.36	0.59	0.0	2.42	2.18
Methyl Bay (flooded forest)	—	—	—	—	—	—	—	—	—	—	—
Methyl Bay (offshore)	—	—	51.0	—	—	49.0	—	—	0.0	—	—
Notigi Reservoir											
Rat Lake	65.90	67.29	48.5	33.70	31.90	50.5	0.39	0.81	1.0	5.72	5.38
West Mynarski Lake	—	—	65.0	—	—	34.0	—	—	1.0	—	—
Central Mynarski Lake	70.85	76.05	90.0	28.86	23.07	10.0	0.29	0.88	0.0	14.9	21.87
Notigi Lake (west basin)	34.65	74.95	91.0	33.43	21.14	9.0	31.91	3.91	0.0	6.85	8.52
Notigi Lake (east basin)	68.90	83.22	95.0	28.83	16.18	4.0	2.27	0.60	1.0	9.58	1.48

were treated with different chemical preservatives appropriate to the analyses to be performed: H_2SO_4 for CH_3Hg^+ and nitrogen (N) determination; HNO_3 + $K_2Cr_2O_7$ for total Hg; HCl for alkali and alkaline–earth metals and organic carbon (org. C); and zinc acetate/acetic acid for sulfide (S^{2-}). For total Hg analyses, duplicate 250-mL subsamples of water mixed with preservative were stored in Pyrex bottles which had been precleaned with HNO_3 and baked at 450°C overnight. Subsamples to be analyzed for dissolved anions were refrigerated, but no preservatives were added.

Plants, soil, and suspended particles collected on membrane filters were oven-dried at ~40°C. Plant samples were pulverized and homogenized with a Cyclone sample mill (UD Corp.).

Chemical Analysis of Water

The temperature, conductivity, and pH of the water were measured at different depths with a temperature–salinity–conductivity meter and pH meter. Total alkalinity (American Public Health Association et al. 1985), dissolved oxygen (O_2) (Stainton et al. 1977), and biochemical oxygen demand (BOD)

TABLE 5. Abundances of minerals in lake sediments and a shoreline deposit of varved Pleistocene silty clay. The values are based on *X*-ray diffraction peak intensities. N.d. = no data.

Sample materials and sampling sites	% of total mineral fraction						% of clay mineral fraction			
	Calcite	Dolomite	Quartz	Potash feldspar	Plagioclase fedspar	Clay minerals	Expandable clay[a]	Kaolinite	Illite	Chlorite
Sediments collected in June 1981										
Granville Lake	6.3	11.7	13.1	5.7	63.0	0.0	0.0	24.2	69.9	5.7
Southern Indian Lake										
Long Bay (shallow cove)	3.5	1.1	7.7	10.4	11.6	65.4	10.7	23.1	64.4	1.6
Long Bay (main basin)	2.2	7.9	14.8	13.6	17.9	43.3	14.2	24.0	55.0	6.6
South Bay	2.4	15.3	16.2	11.2	15.8	38.8	12.1	21.8	58.8	7.1
Notigi Reservoir										
Rat Lake	1.2	3.8	9.2	9.2	10.8	63.4	13.4	24.6	55.4	6.2
Central Mynarski Lake	0.0	0.0	6.4	11.9	14.5	67.1	6.5	18.3	68.1	6.9
Notigi Lake (west basin)	0.0	0.0	6.3	2.9	90.6	0.0	0.0	0.0	0.0	100.0(?)
Notigi Lake (east basin)	4.3	6.5	7.4	11.0	12.5	58.0	7.5	26.4	60.4	5.4
Sediments collected in Aug. 1983										
Granville Lake	0.8	5.1	11.4	11.7	13.5	50.4	15.2	21.3	61.7	1.7
East Mynarski Lake	3.9	4.5	8.3	11.3	14.0	57.7	10.5	26.0	63.4	0.0
Southern Indian Lake										
Long Bay (shallow cove)	0.0	2.7	7.8	3.1	12.8	73.3	14.1	25.1	57.5	3.0
Long Bay (main basin)	3.2	3.3	9.7	9.5	12.8	58.4	13.6	23.5	56.1	6.6
South Bay	3.1	13.4	14.2	9.8	13.5	36.8	12.7	24.2	61.8	1.1
Methyl Bay (flooded forest)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methyl Bay (offshore)	2.6	3.7	12.6	8.8	12.5	52.5	14.0	13.1	60.3	12.4
Notigi Reservoir										
Rat Lake	0.0	5.3	24.6	21.2	30.7	17.9	14.8	22.3	58.7	3.9
West Mynarski Lake	0.0	0.0	7.8	13.1	15.3	56.2	13.8	22.2	59.1	4.7
Central Mynarski Lake	0.0	0.0	9.6	16.0	17.6	56.6	13.8	22.3	56.2	7.6
Notigi Lake (west basin)	3.9	0.5	9.8	11.0	15.4	54.9	12.5	29.1	54.3	3.8
Notigi Lake (east basin)	3.8	1.0	6.7	1.4	11.8	75.0	11.5	27.0	54.8	6.5
Pleistocene silty clay from shore of South Bay (collected in Sept. 1974)	26.0	28.7	17.6	12.1	15.4	0.0	9.9	13.3	61.3	15.3

[a]Includes mixed-layer minerals (e.g. montmorillonite–chlorite and chlorite–vermiculite)

(American Public Health Association et al. 1985) were also determined. Total dissolved Ca^{2+}, Mg^{2+}, N^{+}, K^{+}, Cl^{-}, SO_4^{2-}, and N were estimated by the methods of Stainton et al. (1977) using water passed through 0.45-μm filters. Total water hardness was computed as milligrams dissolved Ca^{2+} + Mg^{2+} per litre. Total and dissolved (<0.45 μm) org. C were determined with a Beckman 915 carbon analyzer. S^{2-}, having been stabilized with zinc acetate/acetic acid solution immediately after sample collection, was reacted with *p*-phenylenediamine dihydrochloride and $FeCl_3$ and determined colorimetrically at 600 nm (cf. Stainton et al. 1977; Strickland and Parsons 1968). Water samples were also analyzed for chlorophyll *a* (Stainton et al. 1977).

The water was analyzed for total Hg by digestion with H_2SO_4/$KMnO_4$/$K_2S_2O_8$ at 95°C, reduction of dissolved Hg^{2+} to Hg^0 vapour with $SnSO_4$, and determination of the Hg^0 by flameless atomic absorption spectrophotometry (AAS) using a Perkin-Elmer 403 AAS unit (Mercury Sampling and Analysis Review Committee 1977). CH_3Hg^+ was extracted with $CuSO_4$ and $NaBr/H_2SO_4$ solutions and benzene, purified by $Na_2S_2O_3$ and KI treatments and accompanying polar/nonpolar solvent fractionations, and determined by gas chromatography (GC) using a Tracor Micro-Tek (MT) 220 GC unit with a Varian electron-capture detector (Uthe et al. 1972). GC analyses were accompanied by confirmation tests employing Ag_2SO_4 to correct for interference (Jensen 1969). Eight-litre water samples were required for CH_3Hg^+ analysis, and since it was not feasible to collect 8 L of whole water in addition to the water collected for centrifugation, only the centrifuge supernate was analyzed for CH_3Hg^+.

The detection limits for total Hg and CH_3Hg^+ in water were 5 ng/L and 0.1–0.2 ng/L, respectively.

Chemical Analysis of Sediments, Suspended Particles, Plants, and Soil

Sediment pH and E_h were measured with a pH meter, using calomel and platinum electrodes for the E_h readings. The sediments, suspended particles, soil, and pulverized plants were then analyzed chemically. The data for sediments and suspended particles were computed on the basis of dry weight as determined by oven-drying at 105°C (in 1981) or freeze-drying (in 1983 and 1984); data for plant and soil samples were computed on the basis of dry weight as determined by oven-drying at 40°C.

Bottom sediments, spun-down suspended solids, plants, and soil collected in 1981 were analyzed for total Hg by digestion with $H_2SO_4/HNO_3/HCl$ and $KMnO_4/K_2S_2O_8$ at 60°C (cf. Mercury Sampling and Analysis Review Committee 1977) followed by flameless AAS. For comparison, the sediment analyses were repeated using HCl/HNO_3 (*aqua regia*) for the extraction. Oven-dried samples were passed through a 100-mesh (150-μm) screen and digested with boiling concentrated HCl/HNO_3 (11:4, v/v) for 15–20 min, and Hg was determined by flameless AAS. HCl/HNO_3 digestion alone was used for determination of total

TABLE 6. Physicochemical properties of water sampled at the surface (s) and near the bottom (b) in 1981 and 1983. Cond. = co

	June 1981											
	pH		Cond. (µS/cm)		O_2 (mg/L)		Temp. (°C)		Ca + Mg (mg/L)		Cond. (µS/cm)	
Sampling site	s	b	s	b	s	b	s	b	s	b	s	b
Granville Lake	7.05	—	49	49	9.70	—	13.5	13.4	8.91	—	101	—
East Mynarski Lake	—	—	—	—	—	—	—	—	—	—	—	—
Southern Indian Lake												
Long Bay (shallow cove)	7.2	—	65	81	9.68	—	17.5	13.0	12.3	—	114	130
Long Bay (main basin)	—	—	66	91	—	—	17.5	12.1	—	—	—	—
South Bay	7.4	—	63	71	10.2	—	15.0	10.5	11.9	—	122	142
Methyl Bay (flooded forest)	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Bay (offshore)	—	—	—	—	—	—	—	—	—	—	—	—
Notigi Reservoir												
Rat Lake	7.2	—	71	60	9.76	—	13.1	7.1	15.0	—	121	142
West Mynarski Lake	—	—	—	—	—	—	—	—	—	—	—	—
Central Mynarski Lake	7.6	—	107	92	9.58	—	14.6	7.9	21.9	—	294	317
Notigi Lake (west basin)	6.8	6.9	58	61	9.32	—	12.5	8.5	12.6	—	120	128
Notigi Lake (east basin)	7.0	6.5	63	76	10.2	8.81	10.8	6.2	14.5	15.1	122	110

TABLE 7. $NH_2OH \cdot HCl$- and citrate/dithionite-extractable and total Mn and Fe content and molar Mn/Fe ratios of sediments collected in 1981 and 1983. Except where oth

	$NH_2OH \cdot HCl$-extractable fraction									Citrate/dithionite-extractable fraction					
	Mn (µmol/g)			Fe (µmol/g)			Mn/Fe ratio × 10^3			Mn (µmol/g)			Fe (µmol/g)		
Sampling site	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983
Granville Lake	2.64	—	51.0[a]	0.840	—	17.7	3390	—	2910[b]	2.60	—	14.5[c]	65.0	—	182[d]
East Mynarski Lake	—	—	33.7	—	—	7.83	—	—	4300	—	—	1.29	—	—	98.5
Southern Indian Lake															
Long Bay (shallow cove)	0.269	0.420	2.04[f]	6.86	7.04	19.5[f]	39.4	59.7	105[f]	0.235	0.353	0.550[f]	57.3	60.7	67.6[f]
Long Bay (main basin)	0.983	—	31.5	7.14	—	5.58	138	—	4820	0.810	—	2.52	61.4	—	149
South Bay	1.38	1.31	3.91[f]	6.75	7.32	5.89[f]	204	179	664[f]	0.963	1.17	1.21[f]	59.8	74.7	54.3[f]
Methyl Bay (flooded forest)	—	—	4.51[f]	—	—	19.9[f]	—	—	227[f]	—	—	0.965[f]	—	—	83.6[f]
Methyl Bay (offshore)	—	—	7.68[f]	—	—	10.9[f]	—	—	704[f]	—	—	2.96[f]	—	—	94.3[f]
Notigi Reservoir															
Rat Lake	2.77	3.01	48.0[f]	10.9	8.77	12.6[f]	257	344	3830[f]	2.42	2.49	15.2[f]	117	93.7	156[f]
West Mynarski Lake	—	—	30.7	—	—	4.81	—	—	6390	—	—	0.879	—	—	95.5
Central Mynarski Lake	0.983	1.33	44.6	10.2	12.2	6.70	98.5	109	6660	0.872	1.32	1.67	108	115	149
Notigi Lake (west basin)	3.82	3.71	58.9	3.26	3.81	21.4	1120	978	2770	3.86	3.60	6.65	175	188	184
Notigi Lake (east basin)	1.33	1.82	4.74	14.6	14.7	25.5	91.0	124	198	0.983	1.01	0.816	131	130	114

[a]Median (mean = 37.9).
[b]Median (mean = 2270).
[c]Median (mean = 10.1).
[d]Median (mean = 143).
[e]Median (mean = 56.8).
[f]Based on analysis of a single sample.

ıd. = conductivity; Temp. = temperature; Ca + Mg = hardness; — = no data.

August 1981						August 1983						
Cond. (µS/cm)		O_2 (mg/L)		Temp. (°C)		pH		Cond. (µS/cm)		O_2 (mg/L)	Temp. (°C)	
s	b	s	b	s	b	s	b	s	b	b	s	b
01	—	8.51	—	19.2	—	6.9	7.25	90	120	9.75	19.5	20.0
–	—	—	—	—	—	8.0	7.05	405	420	2.60	20.0	12.0
14	130	8.12	—	18.2	18.2	6.7	7.2	90	90	8.25	21.5	18.5
–	—	—	—	—	—	7.2	7.3	100	120	8.15	21.0	21.0
22	142	8.78	—	20.0	19.6	7.2	7.0	85	90	9.45	19.0	18.0
–	—	—	—	—	—	6.9	6.5	80	80	8.45	20.0	19.5
–	—	—	—	—	—	6.8	6.8	85	100	8.90	20.5	16.5
21	142	8.50	—	18.9	11.9	7.5	6.9	390	395	2.60	20.0	14.0
–	—	—	—	—	—	8.5	7.2	155	240	1.55	20.0	14.0
94	317	8.99	—	20.2	10.0	7.7	7.0	165	175	3.55	20.0	13.0
20	128	7.89	—	19.0	8.9	7.45	6.95	90	95	4.45	18.0	12.0
22	110	9.11	4.52	19.9	7.0	7.5	6.7	475	455	0.75	21.0	8.0

pt where otherwise noted, the data are means for duplicate or triplicate analyses. — = no data.

able fraction				Bulk composition of sediment								
)	Mn/Fe ratio × 10^3			Mn (µmol/g)			Fe (µmol/g)			Mn/Fe ratio × 10^3		
Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983
182[d]	40.3	—	79.7[e]	13.0	—	83.2	818	—	1022	15.8	—	81.5
98.5	—	—	13.1	—	—	14.1	—	—	701	—	—	20.2
67.6[f]	4.09	5.88	8.13[f]	6.78	5.93	8.83[f]	819	575	796	8.28	10.3	11.1[f]
149	13.1	—	16.6	7.80	—	16.3	853	—	939	9.15	—	17.3
54.3[f]	16.1	15.7	22.2[f]	4.50	10.6	11.6[f]	609	902	611	7.39	11.7	19.0[f]
83.6[f]	—	—	11.5[f]	—	—	10.5[f]	—	—	571	—	—	18.4[f]
94.3[f]	—	—	31.4[f]	—	—	16.7[f]	—	—	695	—	—	24.0[f]
156[f]	20.6	26.7	97.5[f]	10.8	12.7	7.24[f]	1369	598	465	7.92	21.3	15.6[f]
95.5	—	—	9.21	—	—	10.7	—	—	734	—	—	14.7
149	8.04	1.04	11.2	11.1	12.3	14.6	940	1042	892	11.8	11.8	16.4
184	23.2	19.2	36.3	10.4[f]	10.4	38.8	379[f]	1114	1025	27.5[f]	9.30	37.8
114	7.52	7.81	10.6	11.0	12.3	13.7	1041	1014	965	10.6	12.1	14.2

TABLE 8. Integral primary productivity of lake and reservoir environments during the period 18 June – 17 August 1981 (data from Bodaly et al. 1987; R. E. Hecky, pers. comm.) and the chlorophyll *a* content of surface water sampled in August 1983. — = no data.

Sampling site	Primary productivity (g C/m²)	Chlorophyll (μg/L)
Granville Lake	35.8	6.7
East Mynarski Lake	75.5	9.2
Southern Indian Lake		
Long Bay (shallow cove)	27.5	1.3
Long Bay (main basin)	—	2.4
South Bay	14.5	2.4
Methyl Bay (flooded forest)	—	2.4
Methyl Bay (offshore)	—	2.7
Notigi Reservoir		
Rat Lake	—	2.8
West Mynarski Lake	67.9	35.6
Central Mynarski Lake	25.3	8.6
Notigi Lake (west basin)	44.8	4.0
Notigi Lake (east basin)	26.6	3.9

Hg in 1983 and 1984 sediments. CH_3Hg^+ was determined by the same techniques used for water samples, except that toluene was substituted for benzene in the extraction procedure. CH_3Hg^+ extracted from sediments collected in August 1981 could not be determined by GC owing to technical difficulties. Consequently, an alternative method of analysis originally designed for analysis of fish tissue (A. Rieger, Freshwater Institute, Winnipeg, Man., pers. comm.) was employed instead as follows. The CH_3Hg^+, on being extracted into $Na_2S_2O_3$ solution, was digested with $HNO_3/K_2Cr_2O_7/H_2SO_4/KMnO_4$ at 95°C for 30 min, and the total Hg content of the extract was estimated by flameless AAS. Hg determined by this procedure is designated the "total organic (org.) Hg" fraction of the sediment.

The detection limits for total Hg and CH_3Hg^+ in the sediments were 5 ng/g (dry weight) and 0.1–0.25 ng/g (wet weight), respectively. The detection limit for CH_3Hg^+ on a dry weight basis varied with the moisture content of the sediment.

Total iron (Fe), manganese (Mn), and selenium (Se) were determined by AAS techniques following digestion with hot $HNO_3/HClO_4$. Org. C and total N were estimated with a Carlo-Erba CHN analyzer. Sediment samples were also analyzed for S^{2-} using a modification of the method employed for water: fresh sediment was digested with 6 *M* HCl at 60°C to convert the S^{2-} to H_2S gas, which was entrained by a stream of N_2 and bubbled through a zinc acetate solution to precipitate it; the S^{2-} was then determined by the methods used for analysis of water.

Operationally defined forms of Hg, as well as hydrated oxides of Fe and Mn, were selectively extracted from fresh sediment samples with various solvents employed singly or sequentially. The solvent systems and the metal fractions supposedly extracted by them are listed in Table 2. DTPA, HAc, and NaOH extractions were performed on separate subsamples of sediment. The other extractions were performed sequentially in the following order: $CaCl_2 \longrightarrow NH_2OH{\cdot}HCl \longrightarrow$ citrate/dithionite. The extraction methods were adapted from well-known procedures described fully in the literature (see citations in Table 2). Weighed subsamples of sediment were rinsed twice with deaerated (N_2-purged) water and dispersed in the solvents under an atmosphere of N_2 (the ratio of dry weight of sample (grams) to volume of solvent (millimetres) ranging from ~1/100 to 1/2, depending on the extraction method), and the mixtures were shaken mechanically for 2 h. The slurries were then centrifuged, and the supernates were wet-ashed with H_2SO_4/H_2O_2 under ultraviolet (UV) light and analyzed for metals by AAS.

In addition, the humic matter in each sediment sample was estimated. Freeze-dried sediment was dispersed in 0.1 *M* NaOH (in a ratio of 1 g to 20 mL) and shaken for 15 min. Following centrifugation, the org. C content of the supernate was determined by Beckman 915 carbon analyzer, and measurements of the supernate's absorbance at 465 nm and 665 nm (symbolized as A_{465nm} and A_{665nm}) were made with a Bausch and Lomb Spectronic-100 UV-visible spectrophotometer.

All analytical data for sediment extracts were computed on the basis of the unit dry weight of the sediment.

The sediment samples were analyzed for grain size frequency, total organic content, and mineral composition. Freeze-dried 1981 samples were digested wtih hot 27.5% H_2O_2 and wet-sieved with a 4-phi (0.063-mm) screen, after which the fines were dispersed with calgon; grain size frequencies were then estimated with a Sedigraph (Duncan and LaHaie 1979; Sandilands and Duncan 1980). Total organic content was defined as loss of weight on digestion with H_2O_2. The same techniques were used for the 1983 samples except that H_2O_2 treatment was omitted. Semi-quantitative X-ray diffraction analyses were performed on the sediments and Pleistocene silty clay samples with a Philips Automated Powder Diffraction System (PW 1710) employing 40-kW, 20-ma CuKα radiation filtered through Ni. For bulk mineralogy, a layer of randomly oriented sample powder was mounted on a glass slide. For clay mineral analysis, duplicate oriented mounts of the finer than 8 phi fraction of each sample were prepared; one was glycolated and the other was heated at 300–375°C for 1 h, prior to analysis.

Suspended particles collected on membrane filters were digested with concentrated H_2O_2/HNO_3 at ~70°C and analyzed for Hg by flameless AAS.

Experiments

Laboratory assays were performed to measure the ability of sediments to methylate "available inorganic Hg" (defined as readily methylated forms of nonmethyl Hg) under controlled conditions. A modification of an experimental technique developed by Furutani and Rudd (1980) and Furutani et al. (1984) was employed in this phase of the research (see also Hecky et al. 1987; Jackson 1984b, 1987; Ramsey and Ramlal 1987).

The sediment samples used in the experiments were homogenized with a spatula, and 10-g portions were weighed into 125 mL Erlenmeyer flasks, each of which was fitted with a silicone stopper transfixed by a glass tube connected to a three-way stopcock for sampling head gas. The sediment in each reaction vessel was mixed with 20 mL of 10 μ*M* $HgCl_2$ solution (or in some cases plain deionized water) together with whatever additional ingredients were required for the particular experiment, and the composition of the atmosphere in the flask was adjusted as required. The flasks were then incubated for the required length, or lengths, of time in a dark chamber at ~25°C. In most experiments, 0.100 g (or, in a few instances, 0.500 g) of air-dried sphagnum moss pulverized with a Cyclone sample mill (UD Corp.) was added to each flask as a nutrient substrate (sphagnum being a widespread, abundant component of the terrestrial vegetation in northern Manitoba (cf. Hecky et al. 1987)). Freeze-dried green and blue-green algae (*Scenedesmus quadricauda* and *Anabaena variablis*, respectively) were also used as nutrient substrates, 0.020 g of algal cells being added to each

TABLE 9. Total Hg, CH_3Hg^+, and total org. Hg concentrations in sediments collected in 1981, 1983, and 1984. The values are based on analysis of two to seven replicate samples or subsamples except where otherwise noted. Md = median; 0 = not detected; n.d. = no data; — = all individual values are the same.

Sampling site	Total Hg (nmol/g)								CH_3Hg^+ (nmol/g)						Total org. Hg (nmol/g)
	June 1981		Aug. 1981		Aug. 1983		June 1984		June 1981	Aug. 1983		June 1984			Aug. 1981
	Md	Range	Md	Range	Md	Range	Md	Range	Md	Md	Range	Md	Range		
Granville Lake	0.309	0.304–0.374	n.d.	n.d.	0.15	0.10–0.16	0.24	0.21–0.27	0.107[a]	0	—	0	—		n.d.
East Mynarski Lake	n.d.	n.d.	n.d.	n.d.	0.43	0.42–0.46	n.d.	n.d.	n.d.	0	—	0	—		n.d.
Southern Indian Lake															
Long Bay (shallow cove)	0.533	0.176–0.533	0.540	0.457–0.623	0.20	0.19–0.21	n.d.	n.d.	0.0603[a]	0.0010[a]	n.d.	n.d.	n.d.		0.0952[a]
Long Bay (main basin)	0.377	0.264–0.427	n.d.	n.d.	0.16	0.16–0.18	n.d.	n.d.	0.0729[a]	0	0–0.0011	n.d.	n.d.		n.d.
South Bay	0.313	0.305–0.338	0.271	0.240–0.319	0.14	0.13–0.14	n.d.	n.d.	0.0565[a]	0[a]	n.d.	n.d.	n.d.		0.0703[a]
Methyl Bay (flooded forest)	n.d.	n.d.	n.d.	n.d.	0.33	0.32–0.34	0.31	0.21–0.35	n.d.	0.00914[a]	n.d.	0	—		n.d.
Methyl Bay (offshore)	n.d.	n.d.	n.d.	n.d.	0.19	0.17–0.21	0.20	0.18–0.25	n.d.	0.0026[a]	n.d.	0	—		n.d.
Notigi Reservoir															
Rat Lake	0.548	0.443–0.568	0.578	0.508–0.598	0.15	—	n.d.	n.d.	0.0897[a]	0[a]	n.d.	n.d.	n.d.		0.169[a]
West Mynarski Lake	n.d.	n.d.	n.d.	n.d.	0.37	—	n.d.	n.d.	n.d.	0	—	n.d.	n.d.		n.d.
Central Mynarski Lake	1.05	1.03–1.31	1.28	1.07–1.40	0.36	0.36–0.37	n.d.	n.d.	0.0982[a]	0	—	n.d.	n.d.		0.266[a]
Notigi Lake (west basin)	0.155[a]	n.d.	0.130	0.0693–0.251	0.36	0.34–0.37	n.d.	n.d.	0.172[a]	0.0016	0–0.0038	n.d.	n.d.		1.02[a]
Notigi Lake (east basin)	0.618	0.598–1.02	1.08	1.06–1.19	0.36	0.26–0.34	n.d.	n.d.	0.164[a]	0.0015	0–0.0018	n.d.	n.d.		0.798[a]

[a]Based on analysis of a single sample.

flask. The atmosphere in each flask was commerical grade (99.9% pure) N_2 or air, or occasionally H_2S. In many experiments, $CaCO_3$ (1 g/flask) was used as a pH buffer. The experiments were performed under sterile conditions, although it was not feasible to collect the sediments aseptically in the field. All apparatus and all solid ingredients added to the sediment–water slurries were autoclaved, while the solutions were sterilized by filtration.

Two kinds of experiment were performed: (1) incubation of sediments from all field sites in an atmosphere of N_2 with and without added nutrient substrates under identical conditions for the same length of time (10 d) in order to compare the relative methylating capabilities (in effect, the relative methylation rates) of sediments representing different field environments and (2) comparison of sets of replicate sediment subsamples from a single site incubated under different conditions (under different atmospheres, with and without added nutrients, clay, etc.), and usually for varying lengths of time, for the purpose of studying effects of specific environmental factors on methylating capabilities.

After incubation, the head gas in each flask was sampled with a syringe and analyzed for carbon dioxide (CO_2) and methane (CH_4) with a Basic GC unit (CA Instruments, model 8000). The pH and E_h of the slurry were then measured and analyzed for CH_3Hg^+ using the methods described above (including confirmation with Ag_2SO_4). The quantity of CH_3Hg^+ produced in each flask during incubation was computed as nanograms per gram (dry weight) of sediment (for comparison of sediment samples from different sites) or as total nanograms per flask (if sediment from only one field site was used). The "Hg methylating capability" of a sediment sample is here defined as CH_3Hg^+ generated per unit dry weight on incubation under N_2 for 10 d in the presence of $HgCl_2$ under the conditions described above.

The methylation experiments, together with experiments on demethylation of CH_3Hg^+, are treated comprehensively elsewhere (Jackson 1987). Only certain key experiments will be dealt with here.

Data Processing

Relationships between covariant sets of data were subjected to regression analysis. Correlation coefficients (r) and significance probabilities (P) were computed for linear, log–log, semilog, parabolic, and hyperbolic regression lines, and the regression formulas yielding the highest r and P values were selected as the nearest approximations of the trends.

Results and Discussion

Environmental Setting: A Survey of Geographic Patterns of Variation

This section describes the environmental backdrop of the Hg problem, differentiating between possible effects of hydroelectric development and environmental factors unrelated to the impoundment and diversion of the Churchill River. A systematic increase or decrease in the measured values of any parameter in the order Granville Lake ⟶ Southern Indian Lake ⟶ Notigi Reservoir lakes (i.e. in the order of increasing postimpoundment rise in lake level and increasing areal extent of flooding (Table 1) and increasing Hg concentrations in fish (Bodaly et al. 1984a; McGregor 1980; Jackson 1987)), or from the Offshore to the Flooded Forest zone of Methyl Bay, is regarded as a possible or probable result of the flooding caused

TABLE 10. Total Hg and CH_3Hg^+ data for water and suspended matter in 1981. Except in the case of one hypolimnion sample (indicated below), all data represent surface water. Values for total Hg in whole water are means for duplicate samples; all other data represent single samples only. N.d. = no data; 0 = not detected.

Sampling site	Total Hg in whole water in June (nmol/1000 L)	CH_3Hg^+ in centrifuged water (nmol/1000 L)		Centrifuged particulate matter				>0.45 μm particulate matter	
				Total Hg in particulate matter (nmol/g)		Total particulate Hg in water (nmol/1000 L)		Total Hg in particulate matter in August (nmol/g)	Total particulate Hg in water in August (nmol/1000 L)
		June	August	June	August	June	August		
Granville Lake	159	1.25	5.15	0.574	1.33	0.583	1.71	0	0
Southern Indian Lake									
Long Bay (shallow cove)	119	0.788	1.84	0.687	1.20	0.788	2.26	0	0
Long Bay (main basin)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
South Bay	134	0.900	1.91	0.196	0.688	0.312	1.92	0	0
Notigi Reservoir									
Rat Lake	127	1.28	2.71	0.565	1.47	0.412	1.92	0	0
Central Mynarski Lake	131	1.34	3.23	0.860	1.65	2.02	1.79	0	0
Notigi Lake (west basin)	141	1.75	0.800	0.384	2.32	0.442	2.13	49.3	286
Notigi Lake (east basin)	107	1.35	4.21	0.374	1.17	0.543	1.64	1.25	26.5
Notigi Lake (*hypolimnion* of east basin)	183	2.55	2.91	0.698	1.67	2.64	6.38	0	0

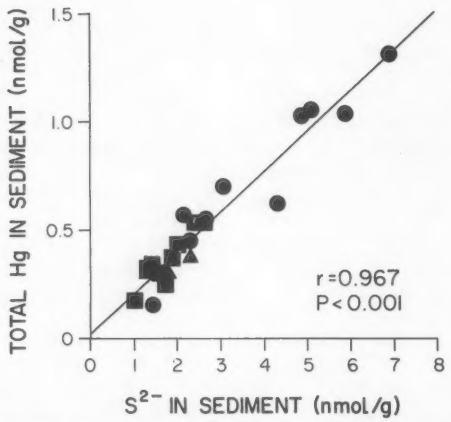

FIG. 2. Relationship between total Hg and nonvolatile S^{2-} in bottom sediments collected in June 1981. ▲, Granville Lake; ■, Southern Indian Lake; ●, Notigi Reservoir lakes.

by the Churchill River diversion. Any gradational change in the order South Bay ⟶ Rat Lake ⟶ West Notigi Lake is viewed as a possible reflection of the introduction of eroded shoreline silt–clay into South Bay and the transport and progressive dilution of this detrital sediment in the downstream direction following diversion of Churchill River flow into the Rat River system by way of South Bay (Fig. 1). Although shoreline erosion had been occurring before the Churchill River diversion, the hydroelectric project not only intensified the process (Hecky and McCullough 1984) but also caused transport of clay and silt from South Bay into the Rat River system; this resulted in a gradational decrease in the abundance of detrital sediment in the order South Bay > Rat Lake > West Notigi Lake (R. E. Hecky, pers. comm.).

Throughout the field area the sediments sampled for this study consisted of grey to brown–grey mud frequently displaying mottling or an approximately 0.5- to 2-cm-thick oxidized surface layer (Table 3) and were composed predominantly of silt and clay (Table 4). Although the samples were commonly odourless, sediments from Methyl Bay (Flooded Forest) and certain samples from Notigi Lake and the Mynarski lakes gave a slight sulfide odour and contained some decaying plant debris or inclusions of black or mixed black and ochre material presumably representing putrefying organic matter. The gross characteristics of the sediments, especially the grey and brown mottling, suggest fluctuation between aerobic and anaerobic conditions, with alternate oxidation and reduction of Fe and only minor amounts of H_2S, if any. This impression is confirmed by the E_h values (Table 3), which range from moderately negative to slightly positive and are generally not far from zero, indicating conditions that are O_2 poor but not extremely reducing. Such conditions are considered ideal for methylation of Hg (Fagerström and Jernelöv 1972; Jackson 1987).

Sediment pH ranged from 6.00 to 6.85 in 1981 and 6.60 to 7.25 in 1983 (Table 3). Most sediment samples contained detectable amounts of calcite and dolomite (Table 5), which presumably exert a buffering effect. These carbonate minerals were undoubtedly derived from the widespread calcareous glacial deposits in the vicinity (Table 5).

Selected physicochemical properties of the water are given in Table 6.

Regional and local anomalies unrelated to impoundment

Granville Lake and West Notigi Lake had a number of anomalies in common. The sediments from those two sites were the only ones in which Mn nodules were found, and among the samples taken in 1981, they had the highest sand and lowest clay concentrations, were richest in plagioclase feldspar but poorest in potash feldspar, and were the only sediments in which expandable-lattice clay (e.g. montmorillonite) was not detected (Tables 4 and 5). These sediments had the highest Mn/Fe ratios, the highest concentrations of Mn oxide extractable with citrate/dithionite or $NH_2OH{\cdot}HCl$ and citrate/dithionite combined, and the lowest $NH_2OH{\cdot}HCl$-extractable Fe content, and their

TABLE 11. S^{2-}, Se, org. C, and N concentrations and N/org. C ratios of sediments collected in 1981 and 1983. All values are means for two, or medians for three or four, replicate samples except where otherwise indicated. — = no data; 0 = not detected.

Sampling site	S^{2-} (nmol/g)			Se (nmol/g)	Org. C (mmol/g)			N (mmol/g)			N/org. C molar ratio		
	June 1981	Aug. 1981	Aug. 1983	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983	June 1981	Aug. 1981	Aug. 1983
Granville Lake	1.81	—	0	5.1	1.1	—	2.25	0.11	—	0.26	0.11	—	0.11
East Mynarski Lake	—	—	1510	8.0	—	—	8.58	—	—	0.889	—	—	0.103
Southern Indian Lake													
Long Bay (shallow cove)	2.52	1.05	0[a]	2.8[a]	1.7	3.0	2.75[a]	0.17	0.18	0.24[a]	0.12	0.060	0.088[a]
Long Bay (main basin)	1.89	—	0	2.0	0.92	—	1.33	0.10	—	0.11	0.12	—	0.086
South Bay	1.42	1.41	0[a]	1.5[a]	0.7	1.4	2.25	0.071	0.082	0.086	0.1	0.060	0.038
Methyl Bay (flooded forest)	—	—	0[a]	4.4[a]	—	—	13.7[a]	—	—	0.59[a]	—	—	0.043[a]
Methyl Bay (offshore)	—	—	2.46[a]	3.2[a]	—	—	2.66[a]	—	—	0.22[a]	—	—	0.0831[a]
Notigi Reservoir													
Rat Lake	2.30	3.22	0[a]	2.0[a]	1.9	2.0	1.83[a]	0.20	0.20	0.14[a]	0.11	0.10	0.78[a]
West Mynarski Lake	—	—	0	7.9	—	—	8.87	—	—	0.939	—	—	0.106
Central Mynarski Lake	5.87	10.7	28.3[b]	7.1	5.2	5.5	6.66	0.66	0.68	0.70	0.13	0.12	0.106
Notigi Lake (west basin)	1.42[a]	1.16	18.2[b]	3.6	1.3[a]	1.7	3.08	0.13	0.13	0.29	0.096[a]	0.60	0.0939
Notigi Lake (east basin)	4.30	4.36	19.8	3.9	3.7	3.3	3.37	0.36	0.39	0.31	0.12	0.12	0.0945

[a]Value for a single sample.
[b]Single value; replicate value was below detection limit.

$NH_2OH{\cdot}HCl$-extractable oxides had the highest Mn/Fe ratios (Table 7). In the 1981 study these two sites were also characterized by the greatest depth of water (Table 1), the lowest surface-water conductivities (Table 6), and the highest values for integral primary productivity (not counting the exceptionally productive East and West Mynarski lakes, which were not included in the 1981 sampling programme) (Table 8). A particularly interesting observation from the standpoint of Hg availability was that the sediments of Granville Lake and West Notigi Lake had the lowest total Hg concentrations (Table 9) whereas the overlying water (unfiltered) had the highest total Hg concentrations (Table 10), suggesting release of solubilized Hg from the sediments.

In 1983, sediments from these lakes showed some comparable anomalies such as aberrantly high Mn/Fe ratios and $NH_2OH{\cdot}HCl$-extractable Mn concentrations (Table 7).

The observations summarized above indicate environments characterized by vigorous flushing, agitation, mixing, and aeration which are conducive to the solubilization of sediment-bound inorganic Hg as well as the deposition of Mn oxide, winnowing out of fine clay particles, and rapid deposition of coarser detritus.

The relatively high-energy main-stream (MS) environments in general had distinctly different physicochemical characteristics than the lower energy backwater (BW) environments. Thus, MS sediments collected in 1981 had higher silt and lower clay concentrations (Table 4) and were richer in $NH_2OH{\cdot}HCl$-extractable Mn (Table 7) than the BW sediments, and the Mn/Fe ratios of their $NH_2OH{\cdot}HCl$-extractable oxides were higher (Table 7). In addition, MS sediments tended to be slightly depleted in HNO_3/HCl-extractable Hg (see Table 14), and associated near-bottom water had lower concentrations of dissolved salts (as measured by conductivity) (Table 6). The relatively coarse, Mn-enriched, somewhat Hg-depleted MS sediments and the relatively dilute MS waters are in keeping with the presumption that higher energy, more rapidly flushed environments occur in the MS than in the BW zone. Differences between MS and BW environments in relation to the mobility, reactivity, and bioavailability of inorganic Hg will be treated fully below and in subsequent papers.

Environmental variations attributable to flooding

Effects of flooding on aquatic and sedimentary environments can be most clearly discerned by comparison of the Flooded Forest and Offshore zones of Methyl Bay and can also be observed by comparison of offshore sites (regions of "old" (preimpoundment) lake bottom) throughout the field area. The role of these environmental alterations in the biogeochemistry of Hg will be treated in full in a later section.

As expected, sediment in the Flooded Forest zone of Methyl Bay contained an abundance of decomposing coarse fragments of recently submerged terrestrial plants, while none were observed in the Offshore zone of Methyl Bay and few or none in other regions of preimpoundment lake bottom (Table 3). In Methyl Bay, therefore, the Flooded Forest sediment is greatly enriched in org. C and N compared with the Offshore sediment (Table 11); and since much of the organic matter is labile, it generates vastly more CO_2 and CH_4 when incubated (Table 12), has a lower E_h (Table 3), and causes a greater degree of O_2 depletion in the overlying water (Table 6). In addition, the Flooded Forest sediment has less $NH_2OH{\cdot}HCl$- and citrate/dithionite-extractable Mn but more $NH_2OH{\cdot}HCl$-extractable Fe, and its extractable oxides have lower Mn/Fe ratios, than is

TABLE 12. Quantities of CH_3Hg^+, and CO_2, and CH_4 generated per unit dry weight by sediments incubated for 10 d under N_2 in the presence of $HgCl_2$ without nutrient enrichment. The sediments were collected in August 1983. — = no data; 0 = not detected.

Sampling site	CH_3Hg^+ (ng/g)		CO_2 (mg/g)		CH_4 (mg/g)	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Granville Lake	—	4.96	0	0.349	0	0
East Mynarski Lake	—	91.1	0	1.81	0	0
Southern Indian Lake						
Long Bay (shallow cove)	—	17.1	0.021	0.469	0	0
Long Bay (main basin)	—	9.25	0.0067	0.178	0	0
South Bay	0	5.11	0.011	0.284	0	0
Methyl Bay (flooded forest)	4.7	741.9	0.036	2.60	0	0.20
Methyl Bay (offshore)	0	5.58	0.011	0.319	0	0
Notigi Reservoir						
Rat Lake	—	11.0	0.010	0.770	0	0
West Mynarski Lake	—	43.3	0.053	1.36	0	0
Central Mynarski Lake	—	20.3	0.017	1.16	0	0
Notigi Lake (west basin)	2.8	23.6	0.011	0.791	0	0
Notigi Lake (east basin)	—	12.8	0	0.405	0	0

TABLE 13. Concentrations of NaOH-extractable org. C and Fe in bottom sediments collected in 1981, together with the A_{465nm} values of NaOH extracts.[a] — = no data.

Sampling site	Org. C (mmol/g)		A_{465nm}		Fe (μmol/g)	
	June	August	June	August	June	August
Granville	0.260	—	0.213	—	14.1	—
Southern Indian Lake						
Long Bay (shallow cove)	0.208	0.271	0.434	0.800	5.04	4.72
Long Bay (main basin)	0.11	—	0.177	—	1.24	—
South Bay	0.052	0.083	0.027	0.053	2.44	4.35
Notigi Reservoir						
Rat Lake	0.291	0.323	0.722	0.735	21.8	20.4
Central Mynarski Lake	0.916	0.520	1.30	0.625	9.78	11.1
Notigi Lake (west basin)	1.35	0.572	3.87	1.77	11.2	12.6
Notigi Lake (east basin)	0.916	0.489	1.69	0.898	18.1	18.3

[a]The org. C and A_{465nm} data are based on analysis of a single sample per site; the Fe data are mean values representing two replicate samples per site.

the case with the Offshore sediment (Table 7), reflecting the relatively O_2-poor conditions imposed by the labile organics in the Flooded Forest zone and, perhaps, a greater abundance of humic–Fe complexes in that region. The Flooded Forest sediment also has a lower N/org. C ratio (Table 11), indicating its abundance of C-rich, N-poor terrestrial organic matter as compared with the more N-rich organic matter derived from aquatic organisms such as algae. Finally, the Flooded Forest sediment had higher concentrations of both total Hg and CH_3Hg^+ and produced more CH_3Hg^+ on incubation with added $HgCl_2$ solution than did Offshore sediment (Tables 9 and 12).

Among offshore sites throughout the field area, the following physicochemical parameters tended to increase in the order Granville Lake ≤ Southern Indian Lake < Notigi Reservoir lakes (i.e. in the order of increasing postimpoundment rise in lake level and in Hg content of fish): (1) *1981 samples*: the org. C, N, S^{2-}, NaOH-extractable Fe, total Hg, and HNO_3/HCl^-, $CaCl_2^-$, and DTPA-extractable Hg concentrations of the sediments (Tables 9, 11, 13, 14) and the A_{465nm} value of the NaOH extract (Table 13), as well as the org. C content (Table 15) and hardness (Table 6) of the water. (2) *1983 samples*: the org. C, N, total Hg, and CH_3Hg^+ content of the sediment (Tables 9 and 11), the quantities of CH_3Hg^+ and CO_2 produced per unit weight of sediment on incubation with $HgCl_2$ solution (Table 12), and the pH of surface water and the conductivity of both surface and bottom waters (Table 6). In 1983, sediment E_h and the O_2 content of bottom water decreased in the order Granville Lake ≥ Southern Indian Lake > Notigi Reservoir lakes.

As would be expected, these data indicate a tendency toward increasing concentrations of organic matter (both labile and refractory (i.e. humic)), and therefore increasing microbial activity, with increasing extent of postimpoundment flooding. Much of the organic matter is undoubtedly derived from recently drowned land. The water hardness data strongly suggest diffusion of Ca^{2+} and Mg^{2+} into impounded water from calcareous glacial drift in the flooded areas. Other major ions (Na^+, K^+, Cl^-, and SO_4^{2-}) did not show the same distribution (T. A. Jackson, unpubl. data). The pH data for surface water may reflect either buffering by the carbonates or variations in the intensity of the photosynthetic activity of phytoplankton. The relevance of these observations to the Hg problem will be discussed below.

TABLE 14. Concentrations of $CaCl_2$-, DTPA-, and HNO_3/HCl-extractable Hg in bottom sediments collected in 1981. $\bar{X}$ = mean; n.d. = no data; — = both individual values are the same.

Sampling site	$CaCl_2$-extractable Hg (nmol/g)[a]				DTPA-extractable Hg (nmol/g)[a]				HNO_3/HCl-extractable Hg (nmol/g)[b]	
	June		August		June		August		June	August
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range		
Granville Lake	0.0257	0.0186–0.0328	n.d.	n.d.	<0.005	—	n.d.	n.d.	0.17	n.d.
Southern Indian Lake										
Long Bay (shallow cove)	0.0312	0.0302–0.0321	0.0346	0.0341–0.0352	0.0120	<0.005–0.0239	<0.002	—	0.28	0.31
Long Bay (main basin)	0.0235	0.0225–0.0244	n.d.	n.d.	<0.005	—	n.d.	n.d.	0.27	n.d.
South Bay	0.0225	0.0186–0.0253	0.0300	0.0299–0.0301	<0.005	—	<0.002	—	0.25	0.30
Notigi Reservoir										
Rat Lake	0.0509	0.0347–0.0672	0.0728	0.0700–0.0756	0.0316	0.0310–0.0322	0.0410	0.0404–0.0416	0.38	0.45
Central Mynarski Lake	0.0531	0.0469–0.0593	0.0627	0.0581–0.0672	0.0527	0.0484–0.0570	0.0648	0.0627–0.0669	0.39	0.543
Notigi Lake (west basin)	0.0340	0.0331–0.0349	0.0736	0.0714–0.0578	<0.005	—	<0.002	—	0.29	0.28
Notigi Lake (east basin)	0.0349	0.0151–0.0547	0.106	0.150–0.108	0.0542	0.0532–0.0552	0.104	0.100–0.107	0.598	0.653

[a]Based on analysis of two replicate grab samples per site.
[b]Based on analysis of a single grab sample per site.

TABLE 15. Org. C concentrations in whole water and centrifuged water collected in August 1981. Except in the case of one hypolimnion sample (indicated below), all data represent surface water. The analyses were done on a single sample per site. — = no data.

Sampling site	Org. C (μmol/L)	
	Whole water	Centrifuged water
Granville Lake	750	710
Southern Indian Lake		
Long Bay (shallow cove)	790	750
Long Bay (main basin)	—	—
South Bay	750	670
Notigi Reservoir		
Rat Lake	833	790
Central Mynarski Lake	1080	999
Notigi Lake (west basin)	833	790
Notigi Lake (east basin)	790	750
Notigi Lake (*hypolimnion* of east basin)	999	874

Effects of eroded clay and silt

Dolomite was found to be the most reliable and useful marker-mineral for tracing the distribution of Pleistocene silty clay eroded into South Bay from shoreline deposits and thence transported downstream and redeposited in Rat Lake and West Notigi Lake. Comprising nearly 30% of the shoreline silty clay collected from the shore of South Bay (Table 5), dolomite was sufficiently abundant as well as stable to make up a measureable component of most lake sediments in the region (Table 5); and considering the regional geology, it was deemed unlikely to have been derived from any sources other than local subaerial Pleistocene deposits.

As anticipated, South Bay sediment was anomalously rich in dolomite, and in both 1981 and 1983, the dolomite content of lake sediment decreased in the order South Bay > Rat Lake > West Notigi Lake (Table 5). The same tendency was shown by the calcite, quartz, and silt content of the sediments, the pH of the surface water and sediments, and the O_2 content of the water in 1981 and by sediment E_h values in 1983 (Tables 3–6). Parameters showing progressive *increase* in the order South Bay < Rat Lake < West Notigi Lake were the following: (1) *in 1981*: the sand, clay, NaOH-extractable org. C and colour (light-absorbing material), citrate/dithionite-extractable Mn, and CH_3Hg^+ content of sediments and, in August only, the $CaCl_2$-extractable Hg content, the percent and absolute increase in $CaCl_2$-extractable Hg from June to August, and the Mn/Fe ratio of the $NH_2OH{\cdot}HCl$-extractable oxides; also, the particulate Hg content of the water and CH_3Hg^+ content and CH_3Hg^+/total Hg ratio of water collected in June (Tables 4, 7, 9, 10, 13, 14); (2) *in 1983*: the org. C, N, Se, and $NH_2OH{\cdot}HCl$-extractable Fe content, N/org. C ratio, and CH_3Hg^+- and CO_2-producing capability of the sediment, and the chlorophyll content of the water (Tables 7, 8, 11, 12); (3) *in both 1981 and 1983*: the $NH_2OH{\cdot}HCl$-extractable Mn and citrate/dithionite-extractable Fe concentrations of the sediments (Table 7).

In brief, there is a progressive decline in the abundance of reworked Pleistocene silty clay from South Bay to West Notigi Lake, accompanied by an increase in algal biomass and in the abundance of organic matter (both humic matter and labile non-humic organics, including autochthonous material derived from the aquatic biota) and Mn and Fe oxides, as well as an increase

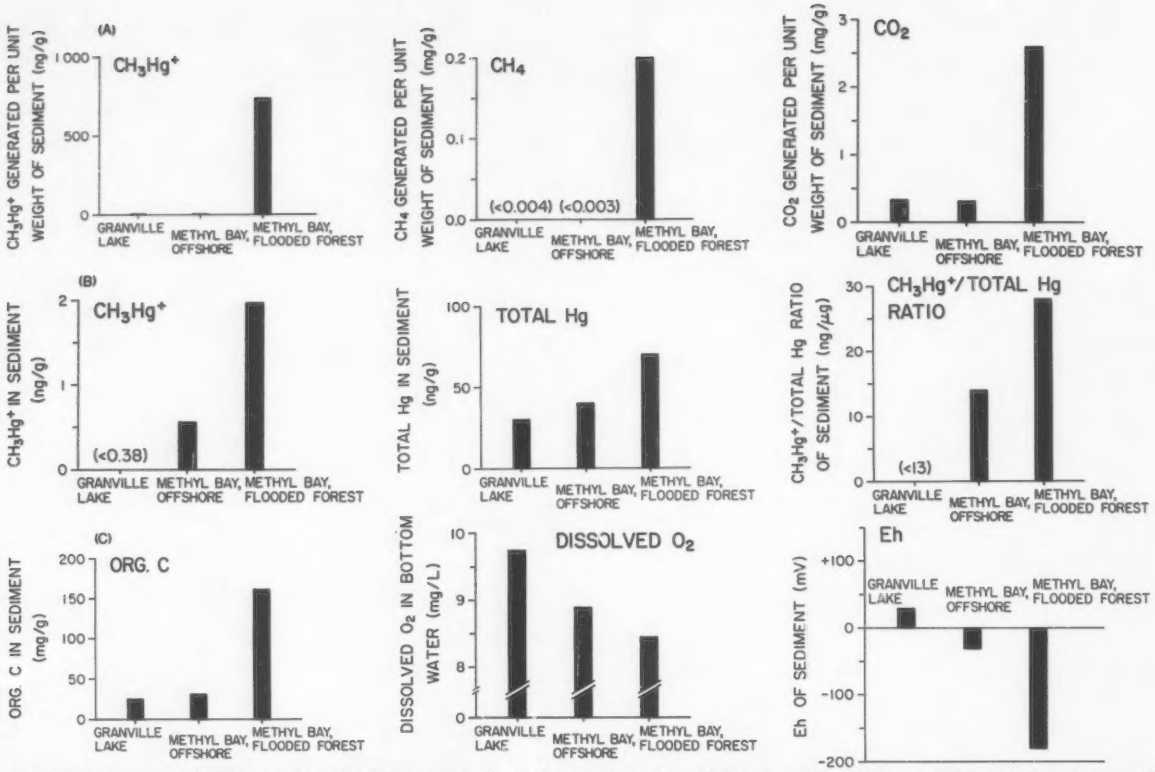

FIG. 3. (A) Amounts of CH_3Hg^+, CO_2, and CH_4 generated by sediments from Granville Lake and the Offshore and Flooded Forest zones of Methyl Bay (Southern Indian Lake) during 10 d of incubation under N_2 in the presence of $HgCl_2$ without nutrient enrichment; (B) CH_3Hg^+ and total Hg concentrations and CH_3Hg^+/total Hg ratios of sediments collected from those sites in August 1983; (C) org. C concentrations and E_h values of the sediments and the O_2 levels in the overlying water.

in Hg availability and the Hg methylating capability of the sediments.

Interpretation of these results in terms of possible effects of eroded silt and clay is made difficult by the fact that the post-impoundment rise in lake level and the depth of water at the sampling site happen to increase in the order South Bay < Rat Lake < West Notigi Lake (Table 1). Thus, downstream gradients in the biogeochemical character of the system may be due to a progressive decrease in the effects of clay–silt or an increase in the environmental impact of flooding, or both. For instance, the downstream increase in algal biomass and labile organics may relfect a decrease in the degree of light extinction by suspended mineral particles in the water and a decrease in the dilution of organics by such particles in the bottom sediments, or it may reflect a downstream increase in the quantiative importance of nutrients introduced from flooded land, or both. In another section (see below) an effort will be made to differentiate between these various effects insofar as Hg methylation is concerned.

Summary

Many of the data given in Tables 1–15 reveal marked regional variations which may, with confidence, be attributed to environmental changes caused by impoundment, either because they afford direct comparison of an inundated terrestrial area and an associated "old" lake environment predating impoundment or because they correlate more or less with the degree of postimpoundment flooding. A large increase in the abundance of terrigenous organic matter and the intensity of heterotrophic microbial activity, including Hg methylating activity, are among the more striking effects of impoundment. Nevertheless, variations in local conditions have to some extent obscured these general tendencies. Consequently, not all of the regional variations in the physicochemical properties of the lakes can be expressed as simple functions of the postimpoundment rise in lake level.

Origin, Distribution, and Abundance of the Hg: Diffuse Sources versus Point Sources

Before analyzing the effects of environmental alterations on CH_3Hg^+ production, it would be appropriate to examine geographical variations in the total Hg content of the lake sediments and to consider the probable sources of the Hg. Above all, it is necessary to ascertain whether there are many local point sources of Hg which might have influenced the distribution of CH_3Hg^+.

The total Hg levels in the sediments (Table 9) fall within the range of background values reported for lake sediments in the Canadian Shield and elsewhere (Jonasson and Boyle 1971; Parks 1976; Jackson and Woychuk 1980a, 1980b). The total Hg in sediments collected in June 1981 gave a highly significant positive linear correlation with S^{2-} (Fig. 2) and somewhat weaker correlations with org. C, N, and $NH_2OH{\cdot}HCl$-extractable Fe (Tables 7 and 11). Samples collected in August 1981 gave the same trend, although with more dispersion. The data

TABLE 16. Total Hg concentrations (ng/g) in soil, leaf litter, and terrestrial plants in the vicinity of Southern Indian Lake. In cases in which a range is given, the analyses were done in duplicate; all others were single analyses. N.d. = no data.

Sampling site	Soil and litter			Plants								
	Leaf litter (birch or choke-cherry)	A horizon (humus and coarse plant debris)	B horizon (clay subsoil and roots	Sphagnum moss		Feather moss	Labrador tea	Black spruce	Reindeer lichen	Betula birch	Paper birch	Willow
				Living plant	Decaying subsurface							
Forest on shore of South Bay	0.0402	0.102	0.0559	n.d.	n.d.	n.d.	n.d.	0.0403 (0.0354–0.0452)	n.d.	0.0559	0.0204	0.0558
Forest and bog on shore of Long Bay	n.d.	n.d.	n.d.	0.0928	0.101	0.0648	0.0594 (0.0512–0.0677)	0.0559	0.0186	n.d.	n.d.	n.d.
Forest near south-central shore of Southern Indian Lake	n.d.	n.d.	n.d.	0.0703	n.d.	0.0154	n.d.	0.0104 (0.0094–0.0113)	n.d.	n.d.	n.d.	n.d.

for all sampling sites are tightly clustered about a single regression line.

These results reveal that all of the Hg in the lakes investigated is derived from diffuse background sources, as no anomalies indicative of local point sources are detectable in Fig. 2 (cf. Jackson 1979). Besides, even the highest concentrations are too small to admit the possibility of pollution other than the ubiquitous low-level global fallout from the atmosphere. The regression line, moreover, indicates that the total Hg level drops to nearly 0 nmol/g at 0 nmol S^{2-}/g, suggesting that most of the Hg had the same immediate source as the S^{2-}, namely organic matter of local aquatic and terrestrial origin. The Hg content of lake sediments tends to increase in the order Granville Lake ≤ Southern Indian Lake < Notigi Reservoir lakes and in the order Methyl Bay Offshore < Methyl Bay Flooded Forest (Table 9), probably indicating that most of the regional variation in Hg concentration is due to the intrusion of Hg from diffuse low-level sources in recently submerged terrestrial materials, in particular, organic matter (Tables 3, 11, 16).

Analysis of shoreline soil and vegetation revealed measurable amounts of Hg in all samples (Table 16). The highest levels occurred in the richly organic "A" horizon of forest soil and in both living and partially decomposed sphagnum moss, an abundant, widespread constituent of the local forest and muskeg communities. These results, although sparse, are consistent with the data of other workers (Bodaly et al. 1984a, 1987) and suggest that sphagnum moss and humus are among the principal immediate sources of Hg in the flooded lake basins.

Methylation of Hg and the Distribution of CH_3Hg^+

Effects of flooding

For the purpose of assessing the effects of flooding on microbial CH_3Hg^+ production, it would be particularly instructive to compare analytical data and results of methylation assays for Granville Lake and the Offshore and Flooded Forest sites of Methyl Bay. These three sites represent, in the order indicated, a regular progression from normal preimpoundment conditions to an environment affected to the greatest degree possible by recently drowned soil and vegetation.

The results (Fig. 3; Tables 3, 9, 11, 12) demonstrate strikingly that the flooding of densely forested land bordering the lakes, with incorporation of terrestrial organic matter into aquatic environments, has produced the following effects: (1) abnormally high rates of CH_3Hg^+ production in sediments (i.e. high levels of "Hg methylation capability"), exceeding by two orders of magnitude the values for the Granville Lake and Offshore Methyl Bay sites, accompanied by accelerated CO_2 and CH_4 production indicative of intensified heterotrophic microbial activity (Fig. 3A); (2) elevated concentrations of both CH_3Hg^+ and total Hg (the latter mainly comprising inorganic (nonmethyl) forms of Hg), and an elevated CH_3Hg^+/total Hg ratio reflecting the enhanced methylation rate (Fig. 3B); *and* (3) elevated org. C concentrations accompanied by O_2 depletion and reducing conditions (Fig. 3C). Regression analysis (Table 17) revealed that Hg methylation capability gave highly significant positive correlations with CO_2 production and org. C concentration and a weaker, although significant, positive correlation with N content, an insignificant positive correlation with total Hg, and insignificant *negative* correlations with the N/org. C ratio of the sediment and the chlorophyll content of the water.

When incubated for varying lengths of time under laboratory conditions without nutrient ammendment, Methyl Bay Flooded Forest sediment generated much more CO_2 and two or three

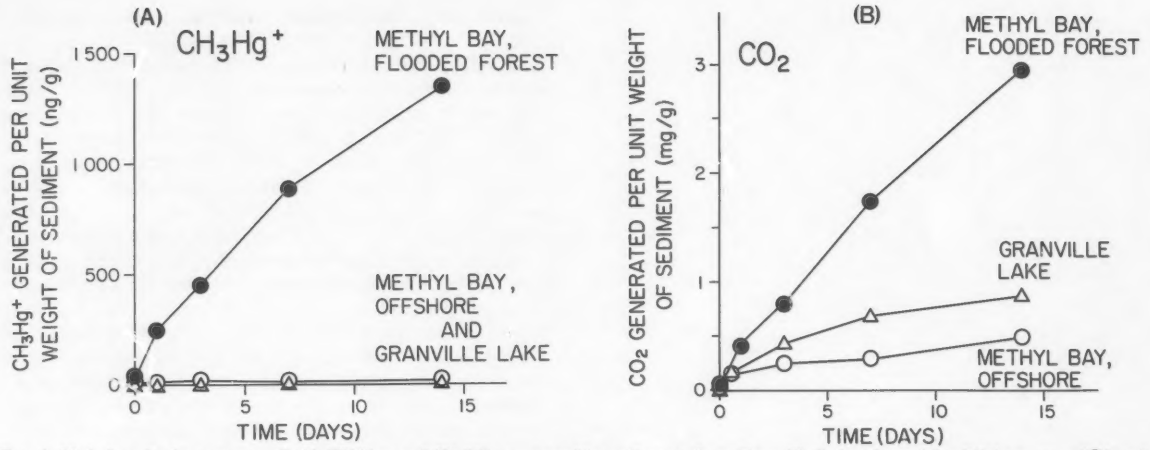

FIG. 4. Variations in the amounts of (A) CH_3Hg^+ and (B) CO_2 generated by sediments from Granville Lake (Δ) and the Offshore zone (○) and Flooded Forest zone (●) of Methyl Bay (Southern Indian Lake) as functions of incubation time during incubation under N_2 in the presence of $HgCl_2$ without nutrient enrichment. The sediments were collected in June 1984.

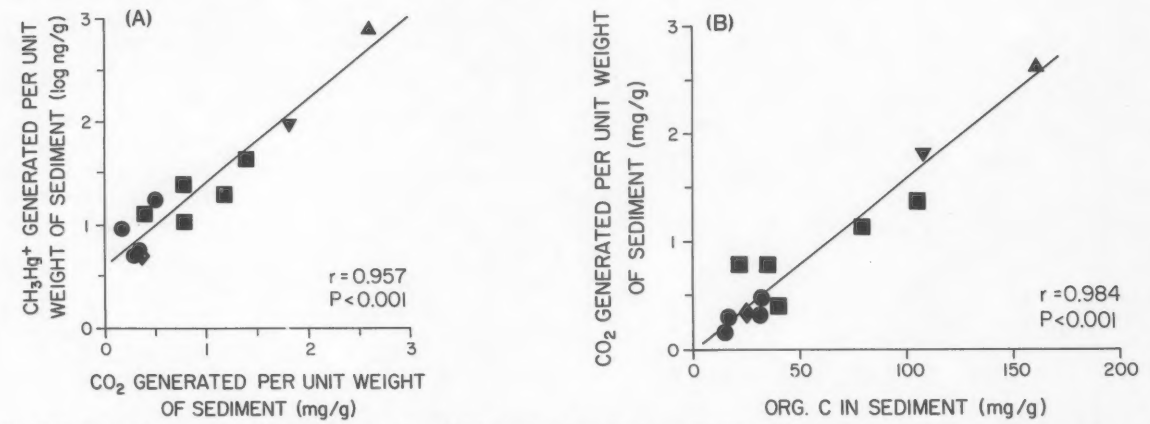

FIG. 5. Relationship between (A) CH_3Hg^+ production and CO_2 production and between (B) CO_2 production and org. C concentration in sediments from different sites. The sediments, which were collected in August 1983, were incubated for 10 d under N_2 in the presence of $HgCl_2$ without nutrient enrichment. ▲, Flooded Forest site in Methyl Bay; ●, other Southern Indian Lake sites; ■, Notigi Reservoir lakes; ♦, Granville Lake; ▼, East Mynarski Lake.

orders of magnitude more CH_3Hg^+ per unit of time than did sediment from either Methyl Bay Offshore or Granville Lake (Fig. 4). When sediments were autoclaved prior to incubation, CH_3Hg^+ production was totally suppressed, proving that the process is entirely dependent on microbial activity.

The data given in Fig. 3 and 4 and in Table 17 lead to the following conclusions. (1) Flooding has caused a huge increase in the rates of Hg methylation in surficial bottom sediments, the rates tending to correlate with the magnitude of the environmental alteration due to flooding. (2) The elevated methylation rates have resulted primarily from stimulation of heterotrophic microbial growth and activity by recently submerged terrestrial organic matter (notably remains of dead plants). The increase in the supply of unmethylated ("inorganic") Hg, which is probably derived from scattered sources in the organic matter, is undoubtedly a contributing factor but is of secondary importance compared with the role of organic matter as a nutrient substrate. Compared with C-rich, N-poor terrestrial organics, the relatively N-rich, C-poor autochthonous organic matter derived from the aquatic biota has had a negligible influence on methylation.

A survey of the methylating capabilities of sediments from all 12 field sites confirmed the conclusion that Hg methylation rates in the study area are controlled by the supply of organic nutrient substrates. Thus, methylation assay data showed a highly significant positive exponential correlation between CH_3Hg^+ and CO_2 generated per unit weight of sediment (Fig. 5A). The CO_2 generated gave, in turn, a strong positive linear correlation with org. C concentration (Fig. 5B). The Flooded Forest site of Methyl Bay showed the highest CH_3Hg^+, CO_2, and org. C levels. The mean rates of CH_3Hg^+ production in offshore sediments increased in the order Granville Lake < Southern Indian Lake < Notigi Reservoir lakes, i.e. in the order of increasing postimpoundment rise in lake level (Fig. 6).

Regression data relating the Hg methylating capability of bottom sediment to other parameters (Table 17) show highly significant positive correlations with the CO_2 production rate and the org. C content of the sediment, significant but weaker

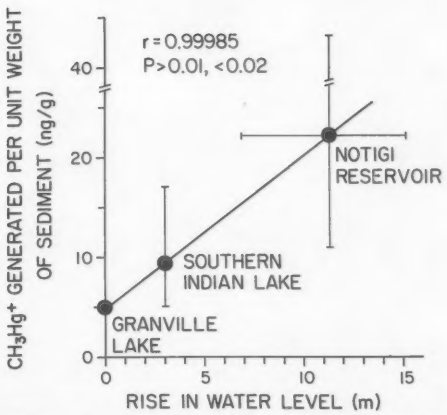

FIG. 6. Relationship between CH_3Hg^+ production by various sediments and the impact of flooding as represented by the rise in water level following impoundment. The points on the plot represent mean values. The sediments, which were collected in August 1983, were incubated for 10 d under N_2 in the presence of $HgCl_2$ without nutrient enrichment.

positive correlations with the total Hg and N concentrations, a significant negative correlation with the N/org. C ratio, and no significant correlation with the chlorophyll content of the water. Repetition of the regression analyses excluding the Methyl Bay–Flooded Forest data, and thus limiting the analysis to offshore sites, led to a startling shift in the values for the correlation parameters (Table 17). N gave almost as strong a correlation as org. C, the N/org. C ratio gave a positive (although statistically insignificant) correlation instead of a negative one, and chlorophyll gave a much stronger correlation (although still weak compared with the correlations given by CO_2 and org. C), and total Hg gave about as strong a correlation as org. C. Comparison of results obtained with and without inclusion of data from the Flooded Forest site reveals that in both offshore and flooded land areas the role of N-rich autochthonous organic matter in stimulating Hg methylation is minor compared with the role of C-rich terrestrial organics but is proportionately much greater, as would be expected, in the offshore zone than in areas of submerged land. The offshore sites gave a strong positive correlation with primary productivity (Table 17), but the relationship depends mainly on the data for the exceptionally eutrophic East and West Mynarski lakes; if those data are excluded, the correlation becomes insignificant ($r = 0.568$; $P > 0.1$).[2] Thus, autochthonous organic matter probably has an important effect on the Hg methylating capability only in those few lakes where the algal biomass is unusually abundant (cf. Jackson 1986). However, a most important fact to note is that in East Mynarski Lake, which had the highest primary productivity (Table 8), the second highest Hg methylating capability (Table 12), and the sediments with the highest Hg content (Table 9) *but was not affected by flooding*, there is no Hg problem. In this lake the sediments contained no detectable CH_3Hg^+ (Table 9), and Hg levels in fish (spottail shiners and yellow perch) have remained at background levels, as in Granville Lake, whereas fish in lakes affected by flooding consistently show considerably higher Hg levels (Bodaly et al. 1987). These observations strongly confirm the conclusion that the Hg problem in northern Manitoba is due to effects of flooding irrespective of other factors (such as an abundance of autochthonous organic matter and Hg) which are potentially conducive to CH_3Hg^+ production.

A correlation matrix relating methylating capability to chemical properties of 1981 sediments (Table 17) provides further striking evidence for the overriding importance of recently submerged terrestrial organics. The NaOH-extractable ("humic") org. C and colour gave much stronger positive correlations than did total org. C or N (even stronger, in fact, than org. C and N in 1983 sediments). Primary productivity gave a very insignificant correlation.

To summarize, the relationships illustrated in Fig. 5 and 6 and recorded in Table 17 strongly support the general conclusion that flooding caused by the Churchill River diversion has resulted in a pronounced increase in rates of CH_3Hg^+ production in sediments and that this increase is due primarily to stimulation of heterotrophic microbial activity by labile organic matter (mostly decomposing remains of plants) in, or from, submerged land areas.

Labile organic matter, however, may affect CH_3Hg^+ production in more ways than one: besides providing nutrient substrates for direct utilization by methylating microbes, it tends to create anoxic conditions (Fig. 3C), and this too affects the activities of these microbes. To differentiate between these two effects, it is necessary to examine the results of experiments in which sediment was incubated with $HgCl_2$ in the presence and absence of an organic nutrient substrate (pulverized sphagnum moss) under atmospheres of air and N_2 and in the presence and absence of sulfide (FeS or H_2S). These experiments are treated more fully elsewhere (Jackson 1987). Briefly, the data (Table 18) reveal that (1) the organic matter promoted methylation of inorganic Hg(II) in the presence and absence of O_2; (2) the net rate of CH_3Hg^+ production was much higher when O_2 was excluded, provided the sulfide concentration was low; and (3) sulfides inhibited or suppressed CH_3Hg^+ production. As sulfide did not suppress CO_2 production, the inhibitory effect of the sulfides on CH_3Hg^+ synthesis can be ascribed to immobilization, and hence diminished availability, of inorganic Hg(II) rather than general suppression of microbial activity. The effect of O_2 may be due to demethylation, which, in the presence of the nutrient substrate, was not inhibited by O_2, and was in fact slightly more active in the presence than in the absence of O_2 (Jackson 1987).

Besides implying that Hg methylation is mediated by different microbial species with widely differing ecological requirements, these experimental results demonstrate that the role of decaying organic matter on flooded land surfaces is twofold, and the two effects are additive: CH_3Hg^+ production is enhanced both by direct utilization of nutrients by methylating microbes and by local O_2-poor conditions created by general heterotrophic activity. The absence of detectable CH_3Hg^+ in East Mynarski Lake despite a relatively large supply of both autochthonous organic matter and inorganic Hg (see above) may be due to the inhibitory effect of sulfide: East Mynarski Lake sediments were anomalously rich in S^{2-} (Table 11).

Other experiments proved that dead cells of aquatic green and blue-green algae as well as moss stimulate methylation (Jackson 1987). These results together with the significant positive correlation between methylating capability and primary productivity (Table 17) show that although terrestrial organic

[2]Exclusion of data for the Long Bay shallow cove site, which was contaminated with nutrients from experimental limnocorrals between the 1981 and 1983 field trips, did not improve the correlation appreciably ($r = 0.591$; $P > 0.1$).

TABLE 17. Correlation matrix relating the Hg methylating capability of sediment (CH_3HG^+ produced per unit dry biological parameters. — = data not computed because not relevant or not capable of being computed.

	CH_3Hg^+ produced by sedim					
	All sites sampled in 1983		All sites sampled in 1983 except Methyl Bay Flooded Forest		All sites sampled in 1981	
	r	*P*	*r*	*P*	*r*	*P*
CO_2 produced by sediment in methylation assay in 1983	0.957	<0.001	0.958	<0.001	—	—
Org. C in 1983 sediment	0.931	<0.001	0.876	<0.001	0.707	0.05
N in 1983 sediment	0.685	>0.01, <0.02	0.865	<0.001	—	—
N/org. C ratio of 1983 sediment	−0.780	>0.001, <0.01	0.505	>0.01	—	—
Chlorophyll in 1983 surface water	0.194	>>0.1	0.563	>0.05, <0.1	0.262	>>0.1
Total Hg in 1983 sediment	0.700	>0.01, <0.02	0.887	<0.001	—	—
% dolomite in 1983 sediment	—	—	−0.559	>0.05, <0.1	−0.792	>0.01, <0.02
% dolomite in June 1981 sediment	—	—	—	—	−0.969	<0.00
Org. C in June 1981 sediment	—	—	—	—	−0.605	>0.1
Org C. in August 1981 sediment	—	—	—	—	0.532	>>0.1
NaOH-extractable org. C in June 1981 sediment	—	—	—	—	0.840	>0.00 <0.01
NaOH-extractable org. C in Aug. 1981 sediment	—	—	—	—	0.889	>0.01, <0.02
A_{465nm} from NaOH extract from June 1981 sediment	—	—	—	—	0.838	>0.00 <0.01
A_{465nm} from NaOH extract from Aug. 1981 sediment	—	—	—	—	0.893	>0.01 <0.02
N in June 1981 sediment	—	—	—	—	0.611	>0.1
N in Aug. 1981 sediment	—	—	—	—	0.504	>>0.1
Primary productivity in 1981 (excluding Rat Lake and main basin of Long Bay)	—	—	—	—	0.568	>>0.1
Citrate/dithionite-extractable Fe in June 1981 sediment	—	—	—	—	0.722	>0.02 <0.05
Citrate/dithionite-extractable Fe in Aug. 1981 sediment	—	—	—	—	0.733	>0.02 <0.05

nit dry weight in a methylation assay) to various chemical, mineralogical and

by sediment in methylation assay in 1983						
tes led 981	Methyl Bay (Flooded Forest and Offshore) and Granville Lake		South Bay, Rat Lake, and West Notigi Lake		Sites sampled in 1981 except Long Bay Shallow Cove	
P	*r*	*P*	*r*	*P*	*r*	*P*
—	0.999997	>0.001, <0.01	—	—	—	—
0.05	0.999947	>0.001, <0.01	0.99907	>0.01, <0.02	0.712	>0.05, <0.1
—	0.9996	>0.01, <0.02	0.999990	>0.001, <0.01	0.685	>0.05, <0.1
—	−0.960	>0.1	0.9998	>0.01, <0.02	—	—
>>0.1	—	—	0.99989	>0.01, <0.02	0.371	>>0.1
—	0.990	>0.05, <0.1	—	—	—	—
>0.01, <0.02	—	—	−0.99934	>0.02, <0.05	−0.797	>0.02, <0.05
<0.001	—	—	−0.984	>0.1	−0.968	<0.001
>0.1	—	—	0.587	>>0.1	0.627	>0.1
>>0.1	—	—	0.542	>>0.1	0.515	>0.1
>0.001, <0.01	—	—	0.999963	>0.001, <0.01	0.927	>0.001, <0.01
>0.01, <0.02	—	—	0.999962	>0.001, <0.01	0.966	>0.001, <0.01
>0.001, <0.01	—	—	0.999986	>0.001, <0.01	0.894	>0.001, <0.01
>0.01, <0.02	—	—	0.999992	>0.001, <0.01	0.892	>0.02, <0.05
>0.1	—	—	0.580	>>0.1	0.640	>0.1
>>0.1	—	—	0.515	>>0.1	0.545	>0.1
>>0.1	—	—	—	—	0.591	>0.1
>0.02, <0.05	—	—	0.9999978	>0.001, <0.01	0.854	>0.01, <0.02
>0.02, <0.05	—	—	0.999968	>0.001, <0.01	0.878	0.05

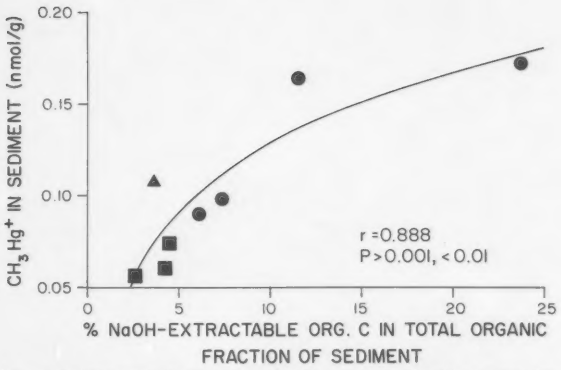

FIG. 7. Relationship between the CH_3Hg^+ content of sediment and the percentage of "humic" (NaOH-extractable) org. C in the organic fraction of the sediment. The samples were collected in June 1981. See Fig. 2 for explanation of symbols.

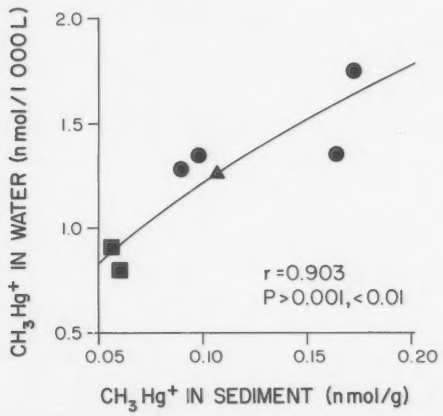

FIG. 8. Relationship between CH_3Hg^+ in centrifuged surface water and CH_3Hg^+ in bottom sediment in June 1981. See Fig. 2 for explanation of symbols.

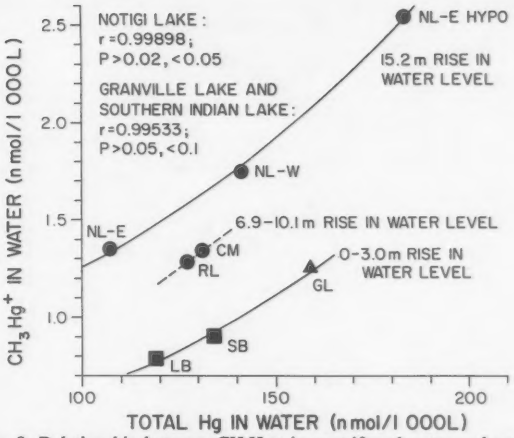

FIG. 9. Relationship between CH_3Hg^+ in centrifuged water and total Hg in whole water in June 1981. GL = Granville Lake; LB = Long Bay (shallow cove); SB = South Bay; RL = Rat Lake; CML = Central Mynarksi Lake; NL-W = West Notigi Lake; NL-E = East Notigi Lake; NL-E HYPO = hypolimnion of East Notigi Lake. See Fig. 2 for explanation of symbols.

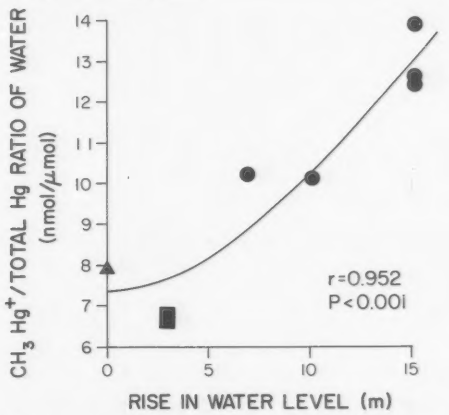

FIG. 10. Relationship between the CH_3Hg^+/total Hg ratio of surface and hypolimnion water in June 1981 and the rise in water level following impoundment and diversion of the Churchill River. The CH_3Hg^+ data represent centrifuged water, and the total Hg data represent whole water. See Fig. 2 for explanation of symbols.

matter is the principal cause of the Hg problem in northern Manitoba, lake productivity is probably a contributing factor.

The CH_3Hg^+ concentrations in sediment samples showed similar trends, and led to the same general conclusions, as data representing the relative rates of CH_3Hg^+ production (Tables 9 and 12). This is demonstrated not only by comparison of the Offshore and Flooded Forest sites of Methyl Bay (Fig. 3A, 3B) but also by comparison of offshore sites representing different degrees of environmental alteration due to flooding (Tables 9 and 12). Thus, in June 1981, CH_3Hg^+ levels were consistently higher in Notigi Reservoir than in Southern Indian Lake, and the highest levels were found in the east and west basins of Notigi Lake, where the impact of postimpoundment flooding was greatest. The only aberration was that the CH_3Hg^+ level in Granville Lake was slightly greater than the values for Southern Indian Lake. CH_3Hg^+ data as such are not available for August 1981, but the "total org. Hg" concentrations in August samples (Table 9) gave a very strong positive correlation with the CH_3Hg^+ concentrations in June samples (r = 0.994 for a log–log plot; $P < 0.001$).[3] In 1983, the pattern of variation shown by CH_3Hg^+ in offshore sediments was comparable with that observed in 1981, the highest values, again, occurring in East and West Notigi Lake (Table 9). Also note that the CH_3Hg^+ content of surface water in June (but not August) 1981 and the total Hg content of suspended particles in August 1981 tended to conform to this pattern (Table 10). These regularities in the distribution of the data in 1981 and 1983 indicate a persistent,

[3]In sediment from West Notigi Lake, the "total org. Hg" unaccountably exceeds the total Hg (Table 9). As this is impossible, there must be an error in the absolute values. Nevertheless, the striking regularities in the data sets and the strong correlations between independent paired data sets such as CH_3Hg^+ in June sediments and total org. Hg in August sediments prove that the patterns of variation are valid. Whatever the error, it would seem to be a systematic one and therefore does not affect the conclusions of this report.

TABLE 18. Production of CH_3Hg^+ from $HgCl_2$ by East Mynarski Lake sediment under experimental conditions in the presence and absence of O_2, organic nutrients (pulverized moss), and sulfides. $CaCO_3$ was used for pH buffereing. The sediment was collected in June 1984. N.d. = no data; 0 = not detected.

	Total CH_3Hg^+ generated (ng)						
	Experiment on effects of aerobic and anaerobic conditions and nutrient enrichment				Experiment on effects of sulfides (with nutrient enrichment)		
	With nutrient enrichment		Without nutrient enrichment		N_2 atmosphere		
Incubation time (d)	N_2 atmosphere	Air atmosphere	N_2 atmosphere	Air atmosphere	No sulfide added	FeS added	H_2S atmosphere
0	140	72	12.6	7.8	17.1	24.5	3.9
1	n.d.	n.d.	n.d.	n.d.	210	125	0
3	856	478	39.1	31.3	1036	450	0
7	1377	449	32.9	34.3	1434	790	0
10	775	343	31.6	33.5	1126	665	0
14	453	291	30.8	34.5	897	642	0

TABLE 19. Correlation matrix relating CH_3Hg^+ in sediments and in surface and hypolimnion water to NaOH- and citrate/dithionite-extractable fractions of the sediments. All data represent samples collected in June 1981.

	CH_3Hg^+ in sediment		CH_3Hg^+/total Hg ratio of water	
	r	*P*	*r*	*P*
Percentage of NaOH-extractable org. C in total organic fraction of sediment	0.888	>0.001, <0.01	0.913	>0.001, <0.01
NaOH-extractable org. C in sediment	0.873	>0.001, <0.01	0.882	>0.001, <0.01
A_{465nm} value of NaOH extract of sediment	0.951	<0.001	0.841	>0.01, <0.02
Citrate/dithionite-extractable Fe in sediment	0.876	>0.001, <0.01	0.967	<0.001

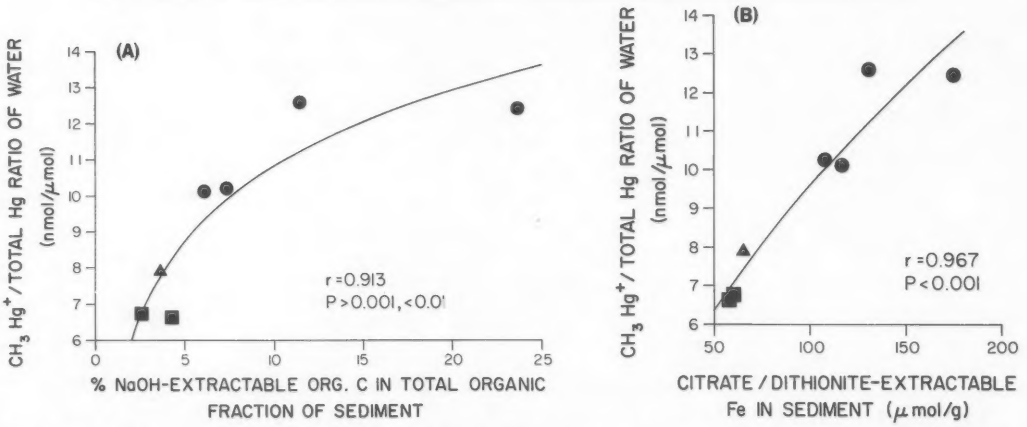
(A)
CH3 Hg+ / TOTAL Hg RATIO OF WATER (nmol/μmol)
r =0.913
P >0.001, <0.01
% NaOH-EXTRACTABLE ORG. C IN TOTAL ORGANIC FRACTION OF SEDIMENT
(B)
CH3 Hg+ / TOTAL Hg RATIO OF WATER (nmol/μmol)
r =0.967
P <0.001
CITRATE / DITHIONITE-EXTRACTABLE Fe IN SEDIMENT (μmol/g)

FIG. 11. Relationships between the CH_3Hg^+/total Hg ratio of surface and hypolimnion water in June 1981 and (A) the percentage of "humic" (NaOH-extractable) org. C in the organic fraction of the sediment and (B) the citrate/dithionite-extractable Fe content of the sediment. The CH_3Hg^+ data represent centrifuged water, and the total Hg data represent whole water. See Fig. 2 for explanation of symbols.

systematic, and significant tendency for CH_3Hg^+ levels to be highest where environmental alteration due to flooding has been greatest.

The CH_3Hg^+ concentration in 1981 sediment was independent of the total (or inorganic) Hg concentration ($r = -0.269$; $P >> 0.1$), but gave significant positive correlations with the NaOH-extractable fraction of the organic matter (expressed as concentration in sediment or as percentage of the total (H_2O_2-digestible) organic matter) and with NaOH-extractable colour and citrate/dithionite-extractable Fe (Fig. 7; Table 19), all of which probably represent the abundance of humic matter. This is consistent with the interpretation that methylation rates and CH_3Hg^+ concentrations in the field area are limited not by the total abundance of inorgnic Hg but by environmental variables affecting the activities of methylating microbes and the availability of the Hg. The relationship between CH_3Hg^+ and humic matter probably signifies that CH_3Hg^+ production rate increases

with increasing abundance of terrestrial organic matter derived from submerged land.

Samples taken in June 1981 (when the lakes were unstratified or weakly stratified) showed a strong positive correlation between CH_3Hg^+ in bottom sediment and CH_3Hg^+ in the overlying surface water (Fig. 8). This implies either that CH_3Hg^+ was diffusing from the sediments into the water or that rates of CH_3Hg^+ production in the water column were proportional to methylation rates in the sediments, or both. The CH_3Hg^+ content of water showed a tendency to increase with increasing total (or inorganic) Hg content (Fig. 9). The trend is moderately significant ($r = 0.798$; $0.02 > P > 0.01$) but is largely masked by effects of environment. The relationship is much more clearly discerned if separate regression lines are constructed for subgroups of sampling regions in each of which the postimpoundment rise in water level (and thus the environmental impact of flooding) has been the same or similar, the variation within each group being no more than 3 m. The plot is thereby resolved into a family of parallel curves displaced vertically with respect to each other because of differences in CH_3Hg^+ concentration per unit concentration of total Hg (Fig. 9). The relative positions of these curves reveal a progressive increase in CH_3Hg^+ in proportion to total Hg with increasing extent of flooding. Consequently, the CH_3Hg^+/total Hg ratio of the water gives a highly significant positive correlation with the rise in lake level following impoundment (Fig. 10). CH_3Hg^+ concentration by itself (not normalized with respect to total Hg) gave a significant but much weaker correlation with rise in lake level: for epilimnion waters only,[4] $r = 0.769$; $0.05 > P > 0.02$. Total Hg alone gave an insignificant negative correlation: $r = -0.479$; $P >> 0.1$. As with CH_3Hg^+ in sediments, the CH_3Hg^+/total Hg ratio of water gave a significant positive correlation with NaOH-extractable org. C and colour and citrate/dithionite-extractable Fe (Fig. 11A, 11B; Table 19).

These results are interpreted as meaning that the rate of CH_3Hg^+ production in the water column or at the sediment–water interface is primarily a function of environmental factors and that the influx of terrestrial organic matter due to flooding has stimulated CH_3Hg^+ production substantially. Variations in the supply of inorganic Hg have evidently been of secondary importance in controlling the rates of methylation. Only within regions where the degree of environmental change due to flooding has been similar does CH_3Hg^+ production appear to vary as a function of total Hg level. Note that CH_3Hg^+ and total Hg correlate much more significantly in the water column than in the sediment. This may be an indication that a higher proportion of the inorganic Hg in the water is available for methylation than is the case with sediment-bound inorganic Hg, perhaps because it is more highly dispersed or is partly in solution.

"Hg methylating capability" and "available inorganic Hg" fraction of sediments: their combined effect on CH_3Hg^+ levels

As demonstrated above, there is a general tendency for *concentrations* of CH_3Hg^+ in sediments and water to increase with an increase in the *rate* at which available inorganic Hg is converted to CH_3Hg^+, the rate being represented by the experimentally estimated "Hg methylating capacity" of the sediment (the amount of CH_3Hg^+ generated per unit weight of sediment when incubated with $HgCl_2$ for a specified period of time under standardized conditions). On close inspection, however, there is, at best, only a very rough covariant relationship. CH_3Hg^+ levels in sediments collected in 1983 (Table 9) give a highly significant positive correlation with the Hg methylating capacities of the sediments (Table 12) ($r = 0.996$; $P < 0.001$),[5] but the relationship exists only because of the extremely high values obtained for the Methyl Bay Flooded Forest site. If the data for this site are excluded so that only offshore samples are taken

[4]In hypolimnion water of East Notigi Lake, CH_3Hg^+ and total Hg levels were anomalously high with respect to epilimnion levels, although the CH_3Hg^+/total Hg ratio was very close to that of epilimnion water.

[5]CH_3Hg^+ concentrations below the detection limit were assigned the value of 0 ng/g in the estimation of the correlation coefficient. Limiting the regression analysis to data for samples containing measurable amounts of CH_3Hg^+ produced nearly the same result ($r = 0.998$; $P < 0.001$).

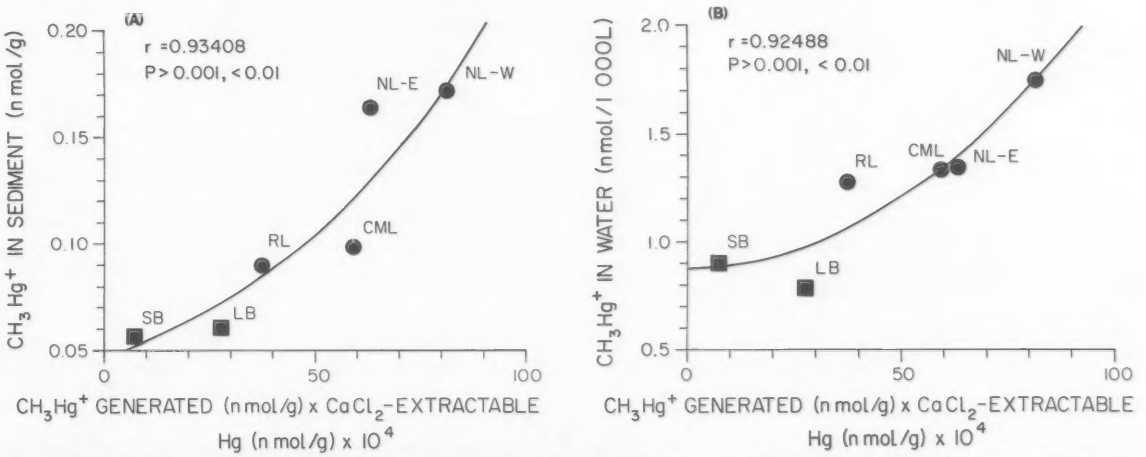

FIG. 12. CH_3Hg^+ concentrations of (A) sediments and (B) centrifuged water collected in June 1981 plotted against the relative rate of sedimentary CH_3Hg^+ production as represented by the "methylating capability" of sediment collected in August 1983 (CH_3Hg^+ generated on incubation with $HgCl_2$) multiplied by the concentration of "available inorganic Hg" in sediment collected in August 1981 ($CaCl_2$-extractable Hg). See Fig. 2 for explanation of symbols. See Fig. 9 for explanation of abbreviations.

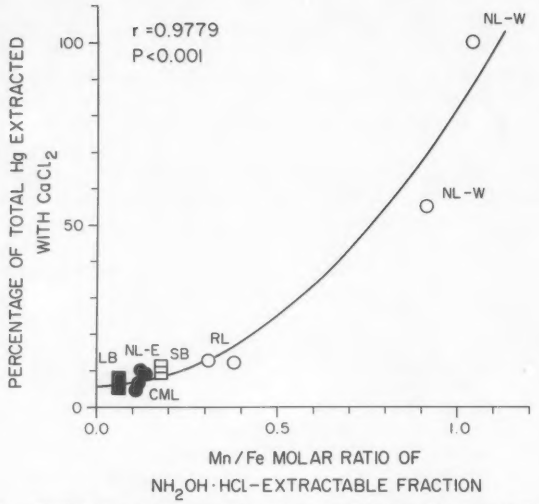

FIG. 13. Relationship between the relative availability of inorganic Hg (estimated as percentage extractable with $CaCl_2$ solution) and the Mn/Fe ratio of the $NH_2OH \cdot HCl$-extractable oxide fraction in sediments collected in August 1981. Open symbols = main-stream sites; solid symbols = backwater sites. ○,●, Notigi Reservoir lakes; □,■, Southern Indian Lake. See Fig. 9 for explanation of abbreviations.

into account, the correlation vanishes ($r = -0.256$; $P >> 0.1$). The Hg methylating capacity gave a moderately significant correlation with the CH_3Hg^+ content of surface water collected in June 1981 ($r = 0.854$; $0.05 > P > 0.02$) but a statistically insignificant, although probably genuine, correlation with the CH_3Hg^+ content of the June 1981 bottom sediment ($r = 0.630$; $P > 0.1$).

Further analysis of the research data revealed that the generally weak correlation between the concentration of CH_3Hg^+ under field conditions and the rate of CH_3Hg^+ production from $HgCl_2$ under laboratory conditions could be explained by failure to take the supply of "available inorganic Hg" into account. The net rate of CH_3Hg^+ production, and hence the concentration of CH_3Hg^+ in sediment or water, depends on two factors: (1) the abundance of "available inorganic Hg" (here defined as forms of nonmethyl Hg that are capable of being methylated, as distinct from the total inorganic Hg pool, which includes unavailable forms) *and* (2) the net rate at which microbes transform this available Hg into CH_3Hg^+ (Bisogni and Lawrence 1975; Beijer and Jernelöv 1979). The Hg methylating capacity represents only the latter.

The available inorganic Hg pool can be seen as comprising all free dissolved Hg^{2+} (Jernelöv 1972; D'Itri 1972; Gavis and Ferguson 1972; Bisogni and Lawrence 1975; Wood 1974) and all weakly adsorbed and weakly complexed Hg^{2+}. Presumably there is a tendency toward attainment of a dynamic steady state between free and bound Hg, the balance shifting back and forth in reponse to external conditions. Thus, sulfides, which bind Hg strongly and suppress the release of Hg^{2+}, decrease the availability of Hg, while oxidizing conditions enhance availability (Jernelöv 1972; Beijer and Jernelöv 1979; Gavis and Ferguson 1972). The net rate at which microbes transform available inorganic Hg into CH_3Hg^+ depends, in turn, on physicochemical variables such as pH, E_h, temperature, salinity, and concentrations of nutrients, O_2, clay-sized minerals, and toxic substances (Jernelöv 1972; Fagerström and Jernelöv 1972; Langley 1973; Bisogni and Lawrence 1975; Shin and Krenkel 1976; Beijer and Jernelöv 1979; Jackson 1984a, 1987). As shown in this paper and elsewhere, anaerobic but sulfide-poor environments enriched in organic nutrient substrates are especially favourable for Hg methylation, although the process is mediated by many different kinds of microbe and therefore occurs over a wide range of conditions (Jernelöv 1972; Fagerström and Jernelöv 1972; Beijer and Jernelöv 1979).

Attempts to demonstrate dependence of CH_3Hg^+ levels in sediments and water on the interaction of the available inorganic Hg supply and Hg methylating capacity of the bottom sediment yielded striking results. Despite the complexity of the biogeochemical cycle of Hg, simple and highly significant empirical relationships were discovered. Thus, the CH_3Hg^+ concentrations of sediments and water collected in June 1981 gave strong positive correlations with the experimentally determined Hg methylating capabilities of the sediments multiplied by the sediments' concentrations of available inorganic Hg as represented by the $CaCl_2$-extractable Hg fraction in samples collected in August (but not June) 1981 (Fig. 12A, 12B). Substitution of "total org. Hg" concentrations in August sediment samples for CH_3Hg^+ concentrations in June sediment samples gave essentially the same result ($r = 0.952$; $0.01 > P > 0.001$). Notigi Reservoir samples gave consistently higher values than Southern Indian Lake samples; no information is available for Granville Lake owing to the absence of $CaCl_2$-extractable Hg data for August. $CaCl_2$-extractable Hg was consistently more abundant in August than in June and showed a somewhat different geographical distribution (Table 14), suggesting that availability was influenced appreciably by seasonal biological activity. Possibly, the release of Hg from decomposing organic matter is most rapid during the summer. In contrast with $CaCl_2$, neither DTPA nor HAc yielded satisfactory results as an extractant for available Hg; this probably indicates that the Cl^- of $CaCl_2$ promotes the extraction of available Hg owing to the formation of soluble complexes.

These findings appear to demonstrate that 0.5 *M* $CaCl_2$ extraction holds promise as a technique for the isolation of available forms of Hg in sediments. The exact forms of the Hg in the $CaCl_2$ extracts are unknown, but the mildness of the reagent suggests that they are weakly bound species, possibly exchangeable ionized Hg(II), as would be expected from their apparent availability. The tentative assumption that the extracted Hg is mostly inorganic (unmethylated) has not yet been tested analytically and remains to be confirmed by further research. Nevertheless, this assumption is consistent with the available evidence. The paired sets of CH_3Hg^+ and $CaCl_2$-extractable Hg data for sediment samples collected in June 1981 did not correlate significantly with each other ($r = 0.340$; $P >> 0.1$), indicating that they represent separate and distinct Hg fractions, even though CH_3Hg^+ levels in June did correlate significantly with $CaCl_2$-extractable Hg levels in August ($r = 0.900$; $0.02 > P > 0.01$). Similarly, previous studies of freshwater environments in northwestern Ontario and in the vicinity of Flin Flon, Manitoba, have failed to show a significant relationship between paired sets of CH_3Hg^+ and $CaCl_2$-extractable Hg data for sediment samples (Jackson and Woychuk 1980b; Jackson 1984a).

At this point, it is necessary to digress briefly and deal with a question of statistical methodology that arises in connection with Fig. 12. Since the plot and the accompanying statistical treatment could be criticized on the grounds that regression analysis employing compound variables may give ambiguous,

misleading, or even spurious results, it should be pointed out that I performed regression analyses employing not only the compound variable but also the individual variables of which this was composed. The results showed that the compound variable gave much stronger correlations ($0.01 > P > 0.001$) than did its individual components (whose P values ranged from $0.02 > P > 0.01$ to $P >> 0.1$). Thus, it is the *combination* of factors representing inorganic Hg availability and Hg methylating capability which is of decisive importance in regulating CH_3Hg^+ production, not any one of the component factors by itself. The correlations shown in Fig. 12 may be regarded as valid for semi-quantitative comparison of the CH_3Hg^+-generating activities of the lakes.

It now remains to ascertain how environmental conditions affect the proportion of the total sediment-bound Hg which is extractable with $CaCl_2$ (i.e. the degree of availability of the inorganic Hg). The abundance of $CaCl_2$-extractable Hg expressed as percentage of the total Hg gave a strong positive correlation with the Mn/Fe ratio of the $NH_2OH{\cdot}HCl$-extractable hydrated oxide fraction of the sediment, and samples from MS regions of vigorous mixing and aeration had higher proportions of $CaCl_2$-soluble Hg and higher Mn/Fe ratios than did samples from BW regions, where the water is less rapidly flushed (Fig. 13). It would seem, then, that aeration enhances the solubility and availability of the inorganic Hg, probably by promoting oxidation of Hg-binding agents such as organic and inorganic sulfides, while fostering a high Mn/Fe ratio owing to precipitation of MnO_2 or MnOOH, which has formed Mn nodules at two of the MS sites. Thus, oxidation–reduction conditions exert two opposing effects on methylation: aerobic conditions enhance the availability of inorganic Hg but depress the methylating capability of the sediment, while anoxic conditions have the reverse effect. In nature there must be a trade-off between these two opposing tendencies. The *net* effect of aeration would be difficult to judge without further research. Note, however, that there is only a slight difference between the CH_3Hg^+ concentrations in sediments from the MS west basin and BW east basin of Notigi Lake (Table 9; Fig. 12), even though there is a large disparity between the inorganic Hg availability data for the same sites (Fig. 13). These data suggest that the two opposing effects of oxidation–reduction conditions very nearly offset one another.

Effects of clay on CH_3Hg^+ production

The experimental data support the conclusion that reworked Pleistocene silty clay from the shore of South Bay tends to inhibit methylation. Thus, the methylating capability of bottom sediment (Table 12) was lower in South Bay than at any other reservoir site and was lower at Rat Lake (the receiving basin for the outflow from South Bay) than in other Notigi Reservoir lakes even though Rat Lake was the scene of the second highest postimpoundment rise in lake level (Table 1). More direct evidence is furnished by correlations between Hg methylating capability and various other biogeochemical parameters in South Bay, Rat Lake, and West Notigi Lake (Table 17). The marker mineral dolomite gave a significant negative correlation, suggesting that the rate of methylation declines with increasing abundance of detrital silt and clay. Comparable results were obtained for the offshore sample sites as a whole (Table 17).

The apparent effect of detrital sediment could be explained by dilution and rapid burial of organic nutrients and by suppression of primary production owing to light extinction, which is known to be an important phenomenon in South Bay (R. E. Hecky, pers. comm.). A third possible role of the silty clay, as suggested by the experimental work of Jackson (1987), is that it greatly promotes demethylation while exerting very little effect on methylation.

Summary

Enlargement of riverine lakes in northern Manitoba to form reservoirs has caused an increase in rates of CH_3Hg^+ production in surface sediments, primarily owing to stimulation of microbial methylation by organic matter from recently flooded land. Utilization of organic nutrients by methylators and consumption of O_2 by general heterotrophic activity have combined to produce this effect. Secondary contributing factors, which are probably of minor importance, include (1) an increase in the supply of inorganic Hg available for methylation owing to the presence of diffuse low-level Hg accumulations in the submerged terrestrial organic matter and (2) production of autochthonous organic matter in the lakes. Silty clay eroded from shoreline deposits appears to depress the net rate of methylation. Aeration in rapidly flushed MS environments enhances the availability of inorganic Hg for methylation but depresses microbial methylating activity; in the more sluggish, less vigorously aerated BW regions, the reverse occurs. The two opposing effects apparently tend to cancel each other.

The existing literature on environmental effects of reservoir creation suggests that the conclusions of this study are widely, if not universally, applicable outside the particular region investigated. Accelerated accumulation of Hg by aquatic organisms appears to be a general, or at least widespread, consequence of impoundment in a wide variety of geographical regions. Therefore, the lessons learned from the Churchill River diversion should be taken into account whenever and wherever reservoir creation is contemplated in the future, especially where fishing is essential to the livelihood of local residents.

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Gene Flow and Genetic Differentiation among California Coastal Rainbow Trout Populations

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Berg, W. J., and G. A. E. Gall. 1988. Gene flow and genetic differentiation among California coastal rainbow trout populations. Can. J. Fish Aquat. Sci. 45: 122–131.

Present levels of genetic variability and estimates of historical rates of gene flow were obtained by analyses of allele frequency data from 31 California coastal rainbow trout populations. Genetic variability was higher than seen in most salmonid species. Seventy-one electrophoretic alleles were segregating at 24 loci. Seven loci were monomorphic. Between-population genetic indentities were high and there was only a weak relationship between geographic proximity of populations and genetic identity. Average within-population heterozygosity and percent polymorphic loci were 0.092 and 35.1, respectively. Estimated total heterozygosity was 0.106; therefore, 86.8% of the total gene diversity could be ascribed to within-population genetic variation. The remaining 13.2% of the total gene diversity represented genetic differences between rainbow trout populations. Both qualitative and quantitative methods of analyses suggested that historical rates of gene flow were high; realized gene flow was estimated to be at least 1.7 migrants per population per generation. It is argued that each population's allele frequencies may simply be expression of temporal fluctuations around the panmictic allele frequencies of the greater global population.

Les niveaux actuels de variabilité génétique et les estimations des vitesses du génique dans le passé ont été obtenus par l'analyse des données sur les fréquences alléliques dans 31 populations de truites arc-en-ciel des côtes de la Californie. La variabilité génétique s'est révélée supérieure à celle que l'on a observée chez la plupart des espèces de salmonidés. L'électrophorèse a permis de mettre en évidence 71 allèles réparties entre 24 loci, dont 7 étaient monomorphes. L'identité génétique entre les populations était élevée et on n'a décelé qu'un faible lien entre la proximité des populations et leur identité génétique. L'hétérozygotie intrapopulation et le pourcentage de loci polymorphes moyens étaient respectivement de 0,092 et 35,1. L'hétérozygotie totale a été estimée à 0,106; par conséquent, 86,8 % de la diversité génétique totale pourrait être attribuée à la variation génétique intrapopulation. Le reste de la diversité génétique totale (13,2 %) correspond aux différences génétiques interpopulations. Les méthodes d'analyse, tant qualitatives que quantitatives, donnent à entendre que les vitesses du flux génique dans le passé étaient élevées; le flux génique réel a été estimé à au moins 1,7 migrant par population par génération. On soutient que les fréquences alléliques de chacune des populations pourraient simplement refléter des fluctuations temporelles autour des fréquences alléliques panmictiques de la population globale plus vaste.

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Rainbow trout, *Salmo (Parasalmo)*[1] *gairdneri* Richardson, range along the west coast of North America from Baja California del Norte to Alaska (Lee et al. 1980). A similar, if not identical form, *S. mykiss* Walbaum, is distributed along the northeastern coast of Asia (Okazaki 1984). The native distribution of the coastal subspecies, *S. g. gairdneri*, in California includes most coastal streams and rivers below major barriers to upstream dispersal, extending to the lower drainages of the Klamath, Sacramento, Pit, and McCloud rivers (McAfee 1966). Since the late 1800s, human intervention has modified this natural distribution by widespread introduction of hatchery-reared stocks and habitat alteration and destruction often resulting in local population extirpation (MacCrimmon 1971; W. Lucoff, California State University, Hayward, CA, unpubl. data).

Over 100 yr ago, Livingston Stone established Baird Station on the lower McCloud River as the first government rainbow trout egg taking station (Needham and Behnke 1962). Baird Station was the original source for most of the hatchery-reared rainbow trout now being used worldwide as both a sport fish and in commercial production (Scott et al. 1978; Busack and Gall 1980). Continued recruitment into established hatchery stocks of rainbow trout from the wild and from other hatcheries (Busack and Gall 1980; Meyer 1983) has resulted in stocks which may be quite different from their founding wild populations. Subsequent plantings of these hatchery stocks in areas

[1]The authors support elevation of *Parasalmo* Valdykov to full generic status.

of naturally reproducing rainbow trout to augment recreational fishing have caused concern about the genetic diversity of native populations (Helle 1981), including possible introgression of nonnative genes into native fish stock. It is, therefore, of primary importance to obtain an appreciation of the distribution of genetic variability within and between extant populations to ensure effective genetic conservation and the continued productivity and future diversity of the fishery.

Of the various methods available for genetic study of population dynamics, starch–gel electrophoresis may be the most valuable. Large data sets of electrophoretically detectable allelic variants are easily obtained and provide raw data needed to characterize the population genetics of a species (Ihssen et al. 1981). Analysis of electrophoretic data can yield detailed estimates of within-population genetic variability, descriptions of genetic similarities between populations, estimates of gene flow among populations, and tests of hypotheses concerning population substructure (Wright 1943, 1969; Nei 1972, 1973; Slatkin 1981). This paper reports the results of such a study of coastal rainbow trout of California.

Materials and Methods

Populations of coastal rainbow trout were sampled at 31 localities in California (Table 1; Fig. 1) known to have been inhabited by anadromous rainbow trout prior to the major water projects of the twentieth century (W. Lucoff, unpubl. data). Nine sampling sites were selected from throughout the Californian range of the subspecies. Twenty-two sampling sites were selected from Sacramento, Pit, and McCloud River drainages landlocked by Shasta Dam (arrow, Fig. 1) including seven sites from the McCloud River which may have contributed genetic material to the original hatchery stocks (Needham and Behnke 1962; Busack and Gall 1980). Two hatchery stocks (sites 22 and 24) established and maintained from single native populations were also examined.

Electrophoretic methodology followed the procedure outlined by Utter et al. (1974). Protein systems were assayed from blood, eye, heart, liver, and muscle (Table 2). Blood was drawn into nonheparinized capillary tubes, centrifuged to isolate plasma, and frozen at −20°C. Solid tissues were mechanically homogenized in an equal volume of 0.25 *M* sucrose and centrifuged at 30 000 × *g* for 20 min. Supernatants were drawn into capillary tubes, sealed, and stored at −20°C. In general, the histochemical staining techniques of Busack et al. (1979) were used. The general peptidase stain was that of Harris and Hopkinson (1976), with each peptidase specifically identified by substrate specificity and differential tissue distribution (Frick 1983).

Enzyme nomenclature followed the recommendations of the International Union of Biochemistry (1984), with locus and allele nomenclature based on suggestions by Allendorf and Utter (1979) and Buth (1983). Electrophoretic patterns were interpreted according to established inheritance models (Allendorf et al. 1975; Utter et al. 1979; Gall and Bentley 1981). Seven duplicated loci examined in this study have not completed rediploidization (Allendorf and Thorgaard 1984) and thus retain alleles of coincident mobility. These duplicated loci were therefore treated as single, pseudo-tetrasomic systems. Aspartate aminotransferase (Aat-1,2) could not be reliably scored from fish which had been frozen prior to tissue preparation. Thus, data for Aat-1,2 was only obtained from 19 of the 31 populations.

Nei's standard genetic identity estimates, *I*, (Nei 1972; Hillis 1984) were computed. The relationship between estimated genetic similarity and geographic proximity among populations was depicted by a phenogram produced by clustering genetic identity values with the UPGMA algorithm (Sneath and Sokal 1973). No infrasubspecific phylogenetic interpretation was intended or implied. Rigorous statistical procedures to assess the accuracy of constructed trees have as yet not been developed, but relative confidence in the phenogram's topology may be inferred by the method of Nei et al. (1983). Based on computer simulation, Nei et al. (1983) suggested that if the product of the shortest branch length, measured as $-\ln I$, and L, the number of loci examined, is greater than or equal to 3, the phenogram may reliably reflect the true relationship among the populations.

Genetic variability was estimated by the proportion of polymorphic loci and by average heterozygosity. A locus was considered polymorphic if the frequency of the most common allele was equal to or less than 0.99. Unbiased heterozygosity esti-

TABLE 1. Locations and sample sizes of coastal rainbow trout populations examined in this study. Site numbers refer to Fig. 1 and *N* is the sample size. Populations 1 through 25 from sites associated with major drainages are indicated as follows: S = Sacramento River; M = McCloud River; P = Pit River; K = Klamath River.

Site (drainage)	*N*	Site (drainage)	*N*
1.(S) Mears Ck.	39	17.(P) McGill Ck.	35
2.(S) Hazel Ck.	31	18.(P) Kosk Ck.	35
3.(S) Shotgun Ck.	14	19.(P) Nelson Ck., main fork	7
4.(S) Slate Ck.	30	20.(P) Pit R., below Pit 4	26
5.(S) Little Squaw Ck., No. Fork	37	21.(P) Rock Ck.	27
6.(M) Chatterdown Ck.	36	22.(P) Crystal Lake Hatchery	52
7.(M) Tom Neal Ck.	29	23.(K) Cold Ck.	25
8.(M) Claiborne Ck.	34	24.(K) Irongate Hatchery	59
9.(M) lower McCloud R.	32	25.(K) Yreka Ck.	15
10.(M) Ladybug Ck.	29	26. Eel R.	47
11.(M) Butcherknife Ck.	33	27. Gualala R.	57
12.(M) McCloud Reservoir	35	28. Feather R., middle fork	12
13.(P) Squaw Ck.	34	29. Big Ck.	21
14.(P) Potem Ck.	19	30. Devil Ck.	20
15.(P) Little Roaring Ck.	33	31. San Luis Rey R.	23
16.(P) Iron Canyon Ck.	36		

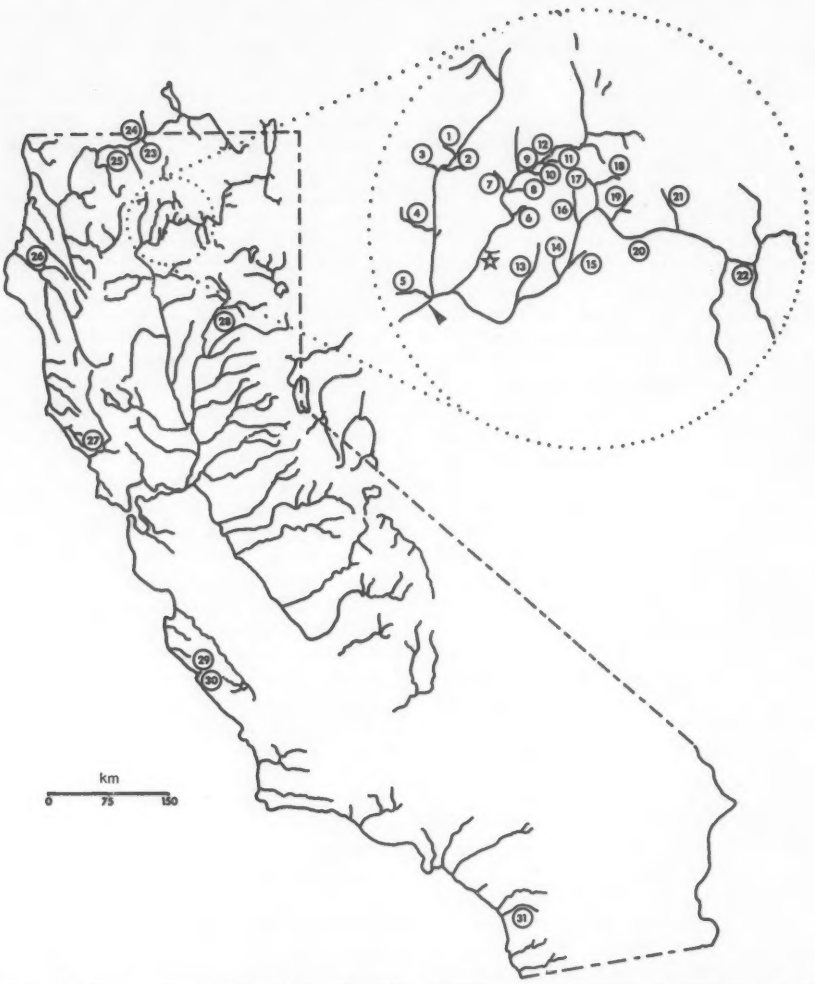

FIG 1. California waterways depicted approximately as they were prior to the major water projects of the twentieth century (W. Lucoff, unpubl. data). Numbers refer to populations of coastal rainbow trout examined in this study (Table 1). Inset is of the upper Sacramento River, McCloud River, and lower Pit River before closure of Shasta Dam (arrowhead) in 1944. Star locates position of Baird Station (see text).

mates and their sampling variances were obtained for each sample from

$$(1) \quad H = \Sigma H_i/L$$

and

$$(2) \quad V(\bar{H}) = \Sigma (H_i - H)^2/L(L\text{-}1)$$

where H_i is the heterozygosity at the ith locus assuming panmixis (Nei and Roychoudhury 1974). Average heterozygosity estimates, H_T, were calculated for each polymorphic locus using allele frequencies averaged over all populations.

The impact of the closure of Shasta Dam in 1944 and the resultant isolation of potentially anadromous rainbow trout populations (W. Lucoff, unpubl. data) was assessed using an unweighted heirarchical gene diversity analysis based on Nei's (1973) method. The 31 sampling sites were divided into two groups. The Shasta group consisted of the 22 populations from drainages landlocked by Shasta Dam. The second group consisted of the nine populations from geographically diverse locations. Aspartate aminotransferase was deleted from the data set used in the gene diversity analysis, as it was scored for only two of the nine populations of the second group. Relative gene diversity among populations within groups (G_{PG}) and among groups (G_{GT}) was estimated as (Nei 1973)

$$(3) \quad G_{PG} = D_{PG}/H_T$$

$$(4) \quad G_{GT} = D_{GT}/H_T$$

where

$$(5) \quad H_T = H_P + D_{PG} + D_{GT}.$$

Historical gene flow among populations was examined using two approaches. The qualitative relationship of the conditional average frequency of alleles to the alleles' incidence, measured as occupancy number, was evaluated using the method pro-

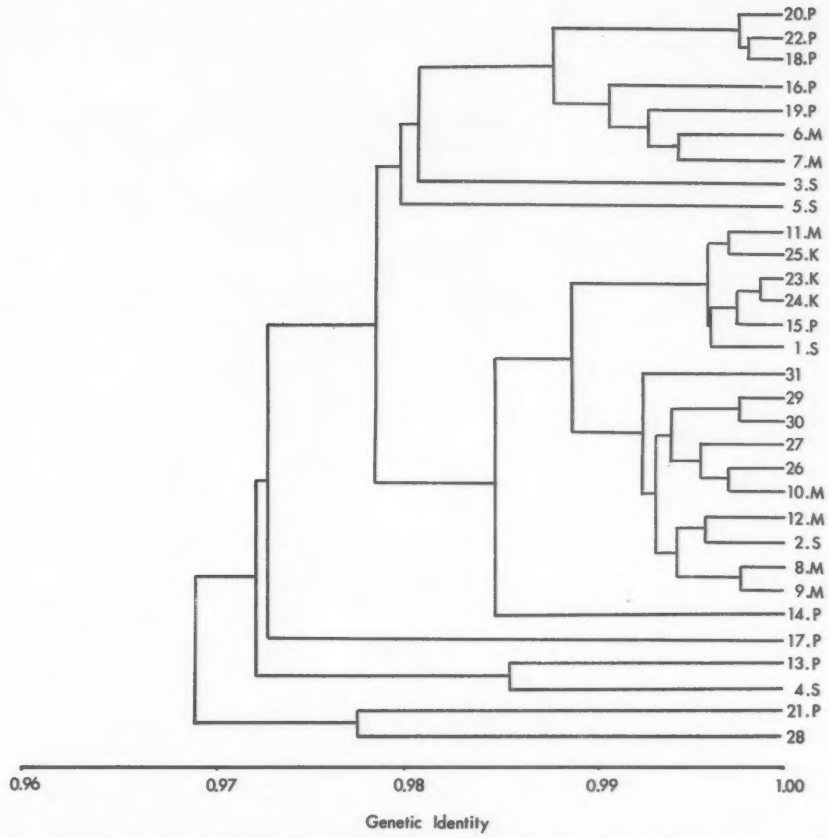

FIG. 2. Phenogram of 31 California coastal rainbow trout populations. Clustering was done with the UPGMA algorithm (Sneath and Sokal 1973) from a matrix of pairwise genetic identity estimates (Nei 1972; Hillis 1984). Overall mean genetic identity = 0.980. Population numbers reference Table 1 and Fig. 1.

posed by Slatkin (1981). The occupancy number of an allele was calculated as i/d where i is the number of populations (demes) in which the allele occurred and d is the total number of populations. Conditional average frequencies, $\bar{p}_i$, were calculated as the unweighted average frequency of all alleles with a given occupancy number. Realized gene flow (N_em_e) was estimated by interpolation from a computer simulation given by Slatkin (1981).

In the second approach, variation of allele frequencies among populations was quantitatively examined using fixation indices (Wright 1943; Nei 1977). A fixation index was calculated for each polymorphic locus as

$$(6) \quad F_{ST} = s_q^2/\bar{q}\,(1 - \bar{q})$$

where $\bar{q}$ and s_q^2 are the mean and sampling variance of allele frequencies, respectively, for all populations. Sampling variances were corrected for sample size as given by Wright (1978). Realized gene flow among populations (N_em_e) was then estimated using the mean F_{ST} value for all polymorphic loci according to the relationshp given by Wright (1969);

$$(7) \quad F_{ST} \simeq 1/(4N_em_e + 1).$$

As shown by Busack (1983, Table 1), this approximation holds over a wide range of values of F_{ST} and N_e as long as $m_e < 0.01$. Although limitations in the application of fixation indices have been identified (Nei et al. 1977), they may provide insight into levels of genetic divergence among populations (Allendorf and Phelps 1981b).

Results

Electrophoretic analysis of 31 populations of rainbow trout detected 71 genetic alleles segregating at 24 variable loci (Table 3). Seven loci were monomorphic: Ck-2, G3pdh-2, Ldh-2, Me-1, Mdh-1,2, Pgm-1, and Pgdh. Allele frequencies are listed in Berg (1987) and are available upon request. At 15 loci, the average frequency of the most common allele was greater than 0.95, while there were five cases in which the most common allele's average frequency was less than 0.80 (Table 3). Additionally, 24 rare alleles ($\bar{q} \leq 0.01$) were detected.

In any particular population, approximately one third of all loci were found to be polymorphic (Table 4). Percent polymorphic loci ranged from 20.7 for Little Squaw Creek (site 5) to 45.2 at Yreka Creek (site 26) with a mean of 35.1. Within population heterozygosity estimates, H, averaged 0.092 (Table 4) and ranged from a low of 0.052 for Mears Creek (site 11) to a high of 0.113 at Chatterdown, Ladybug, and Iron Canyon

TABLE 2. Protein systems studied, Enzyme Commission code number, tissue examined (B = blood, E = eye, H = heart, L = liver, M = muscle), number of loci scored, and quaternary structure.

Protein	Abbreviation	EC No.	Tissue	No. of loci	Quaternary structure
Alcohol dehydrogenase	Adh	1.1.1.1	L	1	Dimer
Glycerol-3-phosphate dehydrogenase	G3pdh	1.1.1.8	M	2	Dimer
L-Iditol dehydrogenase (sorbitol dehydrogenase)	Iddh	1.1.1.14	L	1	Tetramer
Lactate dehydrogenase	Ldh	1.1.1.27	E, L	4	Tetramer
Malate dehydrogenase	Mdh	1.1.1.37	E, H, M	2	Dimer
Malate dehydrogenase (NADP+) (malic enzyme)	Me	1.1.1.40	L, M	3	Tetramer
Isocitrate dehydrogenase (NADP+)	Icdh	1.1.1.42	L, M	3	Dimer
Phosphogluconate dehydrogenase	Pgdh	1.1.1.44	L, M	1	Dimer
Superoxide dismutase	Sod	1.15.1.1	L	1	Dimer
Aspartate aminotransferase	Aat	2.6.1.1	H, M	1	Dimer
Creatine kinase	Ck	2.7.3.2	M	2	Dimer
Phosphoglucomutase	Pgm	2.7.5.1	L, M	2	Monomer
Tripeptide aminopeptidase (substrate: leu-gly-gly)	Lgg (Pep-B)	3.4.11.4	E, M	1	Dimer
Proline dipeptidase (substrate: phe-pro)	Phap (Pep-D)	3.4.13.9	E, M	1	Dimer
Dipeptidase (substrate: gly-leu)	Gl-1 (Pep-A)	3.4.13.11	E, M	1	Dimer
Dipeptidase (substrate: gly-leu)	Gl-2 (Pep-C)	3.4.13.11	E	1	Dimer
Glucosephosphate isomerase	Gpi	5.3.1.9	M	3	Dimer
Para-albumin	Palb	---	B	1	Monomer

creeks (sites 6, 10, and 16). Average heterozygosity values observed for Crystal Lake (site 22), a resident hatchery population, and Irongate (site 24), an anadromous hatchery population, were well within the range for naturally reproducing populations. There was an apparent, although low, correlation between percent polymorphic loci and heterozygosity ($r = 0.39$, $P = 0.03$).

The averaged genetic similarity among populations is depicted in Fig. 2. The most significant observation was the lack of a consistent relationship between genetic similarity and geographic proximity of the populations. The large central cluster with average genetic identity >0.99 (sites 31 through 9) illustrates this random clustering; it includes five populations ranging over 1000 km of the California coast from the San Luis Rey River (site 31) the Eel River (site 26) and five populations located 300 km inland on the Sacramento River (site 2) and McCloud River (sites 8, 9, 10, and 12). Results of accuracy assessment of the phenogram by the method of Nei et al. (1983) suggest very little confidence in the phenogram's topology. Given that the shortest branch is approximately 0.008 distance unit ($d = -\ln I$) in length, over 300 loci would have to be examined before the phenogram could be considered reliable. Indeed the product of the longest branch ($\simeq$0.028 distance unit) times the number of loci used (31) is still much less than 3. These results underscore the high genetic similarity among all the populations examined.

Relative intergroup gene diversity, G_{GT}, averaged only 1.6%, while gene diversity among populations within groups, G_{PG}, accounted for 12.4% of the total (Table 5). With the lone exception of the Icdh-2 locus, G_{GT} values ranged from 0.00 to 2.3%, suggesting little genetic differentiation between the 22 populations taken above Shasta Dam and the nine populations obtained from widely distributed localities. The relatively high G_{GT} value of 9.3% observed at the Icdh-2 locus was a function of two alleles; Icdh-2 (95) was restricted to four coastal populations north of San Francisco Bay, while Icdh-2 (105), although present in populations of both groups, occurred at high frequency in the Shasta group. The high estimates of G_{PG} observed for a few loci were the result of very localized differentiation, characterized by the presence of alleles in only one or a few populations within a group. The within-population component of relative gene diversity ranged from 55.6 to 99.1% of the total, with a mean value of 86.0%.

Since there was little evidence for divergence due to regional separation using Shasta Dam as the point of demarcation, the 31 populations were considered as a single group. Deleting groups from the above analysis and including data for the Aat-1,2 loci yielded an estimated total heterozygosity of 0.106 $\pm$ 0.057. Using the average within-population estimate of heterozygosity, $H = 0.092$, the relative gene diversity attributable to divergence among populations was 13.2%. The remaining 86.8% represented genetic variation within populations.

The observed distribution of conditional average frequencies of alleles to the allele's incidence corresponds very well to that expected for high levels of gene flow (Fig. 3). Interpolation from Slatkin's (1981) fig. 2A suggests that realized gene flow ($N_e m_e$) is on the order of 1.25–2.5. The conditional average frequency calculated for the lowest incidence class was higher than expected from Slatkin's work due to the presence of two unique alleles at high frequencies: Icdh-1 (85), $q = 0.450$, and Gpi-1 (50), $q = 0.417$.

The average fixation index for the 24 variable loci was 0.127 and ranged from 0.002 to 0.422 for individual loci (Table 6). Applying equation (7), the realized gene flow, $N_e m_e$, was calculated to be 1.7, a value similar to that found using Slatkin's graphic method. Weir and Cockerham (1984), in an evaluation of various methods of estimating F_{ST}, suggested that equation (7) would yield modest underestimates of $N_e m_e$. Therefore, $N_e m_e$

TABLE 3. Unweighted averages, standard errors, and ranges of allele frequencies at 24 electrophoretic loci in 31 California populations of coastal rainbow trout. Number of gene copies examined at each locus is listed below locus abbreviation. The allele designated Me-3,4 (100) represents two inadequately resolved Mendelian alleles.

Locus/ no. of genes	Allele	$\bar{q}$	SE	Range	Locus/ no. of genes	Allele	$\bar{q}$	SE	Range
Aat-1,2	(110)	0.0271	0.0026	0.000 – 0.197	Mdh-3,4	(110)	0.0001	<0.0001	0.000 – 0.004
2284	(100)	0.9715	0.0026	0.803 – 1.000	3844	(107)	0.0053	0.0004	0.000 – 0.050
	(90)	0.0014	0.0003	0.000 – 0.022		(100)	0.8885	0.0032	0.650 – 1.000
						(95)	0.0216	0.0015	0.000 – 0.200
Adh	(−100)	0.9817	0.0014	0.814 – 1.000		(85)	0.0510	0.0019	0.000 – 0.213
1922	(−80)	0.0183	0.0014	0.000 – 0.186		(75)	0.0335	0.0018	0.000 – 0.221
Ck-1	(100)	0.9946	0.0006	0.931 – 1.000					
1822	(70)	0.0054	0.0006	0.000 – 0.069	Lgg	(150)	0.0121	0.0008	0.000 – 0.117
					1922	(100)	0.9846	0.0010	0.883 – 1.000
G3pdh-1	(140)	0.0746	0.0045	0.000 – 0.625		(60)	0.0033	0.0004	0.000 – 0.063
1922	(100)	0.9246	0.0044	0.375 – 1.000					
	(85)	0.0008	0.0002	0.000 – 0.026	Gl-1	(120)	0.0525	0.0044	0.000 – 0.714
					1922	(100)	0.9459	0.0044	0.286 – 1.000
Icdh-1	(100)	0.9839	0.0028	0.550 – 1.000		(80)	0.0016	0.0003	0.000 – 0.050
1754	(85)	0.0161	0.0028	0.000 – 0.450					
					Gl-2	(120)	0.0215	0.0015	0.000 – 0.152
Icdh-2	(105)	0.2241	0.0097	0.000 – 0.846	1922	(100)	0.9734	0.0016	0.848 – 1.000
1754	(100)	0.7573	0.0094	0.154 – 1.000		(80)	0.0051	0.0005	0.000 – 0.053
	(95)	0.0186	0.0022	0.000 – 0.237					
					Phap	(120)	0.0063	0.0007	0.000 – 0.097
Icdh-3,4	(170)	0.0521	0.0026	0.000 – 0.370	1922	(100	0.9920	0.0006	0.903 – 1.000
3844	(140)	0.7398	0.0060	0.294 – 1.000		(90)	0.0017	0.0002	0.000 – 0.032
	(100)	0.0641	0.0028	0.000 – 0.365					
	(90)	0.0008	0.0002	0.000 – 0.024	Gpi-1	(150)	0.0010	0.0002	0.000 – 0.017
	(60)	0.1432	0.0036	0.000 – 0.333	1920	(100)	0.9856	0.0025	0.584 – 1.000
						(50)	0.0134	0.0025	0.000 – 0.417
Ldh-1	(150)	0.0025	0.0005	0.000 – 0.079					
1860	(100)	0.9975	0.0005	0.921 – 1.000	Gpi-2	(110)	0.0041	0.0005	0.000 – 0.076
					1922	(100)	0.9942	0.0006	0.924 – 1.000
Ldh-3	(100)	0.0013	0.0002	0.000 – 0.032		(66)	0.0134	0.0003	0.000 – 0.052
1860	(75)	0.9975	0.0003	0.957 – 1.000					
	(35)	0.0011	0.0002	0.000 – 0.025	Gpi-3	(115)	0.0063	0.0012	0.000 – 0.196
					1922	(100)	0.9838	0.0020	0.696 – 1.000
Ldh-4	(100)	0.9837	0.0012	0.862 – 1.000		(85)	0.0078	0.0010	0.000 – 0.150
1922	(75)	0.0163	0.0012	0.000 – 0.138		(60)	0.0021	0.0004	0.000 – 0.065
Me-2	(100)	0.9686	0.0028	0.639 – 1.000	Iddh-1,2	(135)	0.0098	0.0010	0.000 – 0.132
1906	(50)	0.0304	0.0039	0.000 – 0.361	3844	(100)	0.5398	0.0014	0.500 – 0.645
	(0)	0.0010	0.0002	0.000 – 0.032		(40)	0.4504	0.0020	0.280 – 0.500
Me-3,4	(100)	0.9922	0.0005	0.966 – 1.000	Pgm-2	(100)	0.8410	0.0040	0.621 – 1.000
3168	(80)	0.0078	0.0005	0.000 – 0.034	1922	(85)	0.1590	0.0040	0.000 – 0.379
Palb-1,2	(105)	0.4293	0.0055	0.110 – 0.806	Sod	(170)	0.0003	<0.0001	0.000 – 0.009
3844	(100)	0.5707	0.0055	0.194 – 0.890	1922	(140)	0.2028	0.0060	0.000 – 0.662
						(100)	0.7785	0.0056	0.338 – 1.000
						(60)	0.0184	0.0012	0.000 – 0.130

= 1.7 can be considered a minimum estimate of realized gene flow among the 31 populations.

There was little relationship between population differentiation measured as heterozygosity or sampling variance and the fixation index for individual loci. Wright (1978) has suggested that at the intraspecific level, the fixation index is likely to yield more detailed information than measures of absolute differentiation. This situation is demonstrated by results of Icdh-1, Gpi-1, and Iddh-1,2 which had the highest and the lowest F_{ST} values. At the Iddh-1,2 loci, the fixation index was near zero and the sampling variance was low but the heterozygosity was very high. This indicated that all populations examined had comparable levels of genetic variability at the Iddh-1,2 loci. The two loci with the highest F_{ST} values had low estimates of heterozygosity and low to moderate sampling variances. These high fixation indices were the direct result of uncommon alleles occurring in only a few populations at moderately high frequencies.

Alleles that were found in only one or two populations are listed in Table 7. The loci at which these alleles occurred include those with high relative gene diversity values (Table 5), those contributing to the high conditional average frequencies at low incidence values, and those with high fixation indices. Analysis of these uncommon alleles and their usefulness in evaluating levels of recent genetic isolation of individual populations is presented elsewhere (Berg and Gall 1988). However, it is of some interest to note that 17 of 71 or 24% of the alleles observed in this study occurred in only one or two of the 31 populations sampled. Furthermore, the distribution of these alleles among populations is clearly nonrandom. Some populations such as those in Slate Creek (site 4), Eel River (site 26), Gualala River

TABLE 4. Estimates of percent polymorphic loci, %*P*, and heterozygosity for 31 California populations of coastal rainbow trout. Site numbers refer to Table 1 and Fig. 1.

Site	No. of loci examined	%*P*	Heterozygosity	
			H	SD
1	30	40.0	0.082	0.027
2	30	36.7	0.085	0.029
3	30	26.7	0.104	0.037
4	31	41.9	0.108	0.032
5	29	20.7	0.069	0.032
6	30	40.0	0.113	0.034
7	30	33.3	0.097	0.033
8	30	26.7	0.092	0.032
9	30	40.0	0.105	0.032
10	30	36.7	0.113	0.034
11	30	33.3	0.052	0.023
12	31	38.7	0.104	0.030
13	30	33.3	0.104	0.034
14	30	43.3	0.100	0.031
15	30	43.3	0.079	0.026
16	30	43.3	0.113	0.034
17	29	37.9	0.095	0.030
18	30	36.7	0.081	0.025
19	30	36.7	0.107	0.031
20	31	35.5	0.086	0.027
21	30	36.7	0.105	0.033
22	30	33.3	0.078	0.025
23	30	23.3	0.056	0.024
24	31	38.7	0.073	0.024
25	30	33.3	0.083	0.025
26	31	45.2	0.092	0.030
27	31	35.5	0.096	0.030
28	29	27.6	0.098	0.035
29	31	25.8	0.090	0.033
30	31	38.7	0.093	0.032
31	31	25.8	0.086	0.031
Average	30.2	35.1	0.092	0.030

(site 27), and San Luis Rey River (site 31) are each distinguished by the presence of at least three uncommon alleles.

Discussion

All of the populations of coastal rainbow trout we examined were genetically very similar, despite high genetic variability. Maintenance of between population genetic similarity over large geographic distances is undoubtedly due to a high rate of gene flow. A few populations were distinguished by the presence of several unique alleles which may be considered as indicators of localized restriction of gene flow (Berg and Gall 1988). Thus, these rainbow trout populations were characterized by moderate levels of genetic diversity in the midst of overall similarity.

The level of heterozygosity observed was not only higher than most estimates for teleost fishes and vertebrates in general (Kirpichnikov 1981; Avise and Aquadro 1982; Nevo et al. 1984), but was also higher than estimates obtained for other species of Salmonidae (Grant et al. 1980; Okazaki 1982; Vuorinen 1982; Ryman 1983; and references therein). Genetic variability observed for two hatchery populations agreed with earlier findings (Busack et al. 1979; Guyomard 1981; Thompson 1985) indicating little loss of genetic variability in the hatchery environment (but see Allendorf and Phelps 1980; Vuorinen 1984).

TABLE 5. Relative gene diversity estimates for 23 polymorphic loci among 31 California populations of coastal rainbow trout. Populations grouped as those landlocked by Shasta Dam versus all other populations. Averages are based on 30 loci (Aat-1,2 excluded). P = population, G = groups, and T = total.

Locus	H_P/H_T	G_{PG}	G_{GT}
Icdh-1	55.6	43.4	1.0
Gpi-1	61.8	37.2	1.0
Icdh-2	65.5	25.2	9.3
Gl-1	69.5	28.3	2.3
G3pdh-1	71.8	26.8	1.4
Me-2	76.3	21.5	2.2
Gpi-3	83.0	15.0	2.0
Sod	84.6	13.2	2.3
Icdh-3,4	86.6	10.9	0.4
Palb-1,2	88.7	11.1	0.2
Pgm-2	88.9	11.0	0.1
Mdh-3,4	91.3	8.3	0.4
Adh	92.4	7.5	0.1
Ldh-4	93.2	6.7	0.1
Gl-2	92.7	6.3	1.0
Ldh-1	92.3	7.6	0.2
Lgg	93.4	6.5	0.0
Gpi-2	94.8	4.9	0.3
Phap	94.9	4.6	0.5
Ck-1	95.7	4.3	0.0
Ldh-3	97.5	2.2	0.3
Me-3,4	98.3	1.5	0.2
Iddh-1,2	99.1	0.7	0.2
Average	86.0	12.4	1.6

TABLE 6. Estimates of average heterozygosity, sampling variance of allele frequencies, and fixation indices for 24 polymorphic loci among 31 California populations of coastal rainbow trout. Loci are listed in descending order of their fixation index. Average fixation index for the 24 loci was 0.127 and average heterozygosity for all 31 loci studied was 0.106.

Locus	No. of populations	No. of alleles	H_T	s_q^2	F_{ST}
Icdh-1	28	2	0.032	0.0067	0.422
Gpi-1	31	3	0.028	0.0104	0.363
Icdh-2	28	3	0.376	0.1315	0.350
Gl-1	31	3	0.103	0.0321	0.296
G3pdh-1	31	3	0.140	0.0317	0.227
Me-2	31	3	0.061	0.0134	0.219
Gpi-3	31	4	0.032	0.0050	0.157
Sod	31	4	0.352	0.0531	0.151
Aat-1,2	19	3	0.055	0.0066	0.114
Icdh-3,4	31	5	0.425	0.0437	0.103
Palb-1,2	31	2	0.490	0.0243	0.099
Pgm-2	31	2	0.267	0.0113	0.085
Mdh-3,4	31	6	0.206	0.0152	0.074
Adh	31	2	0.036	0.0013	0.072
Ldh-4	31	2	0.032	0.0009	0.058
Gl-2	31	3	0.052	0.0039	0.053
Ldh-1	31	2	0.005	0.0001	0.052
Lgg	31	3	0.030	0.0011	0.036
Gpi-2	31	3	0.012	0.0004	0.035
Phap	31	3	0.016	0.0005	0.034
Ck-1	31	2	0.011	0.0002	0.031
Ldh-3	31	3	0.005	0.0001	0.013
Me-3,4	24	2	0.015	0.0001	0.009
Iddh-1,2	31	3	0.506	0.0009	0.002

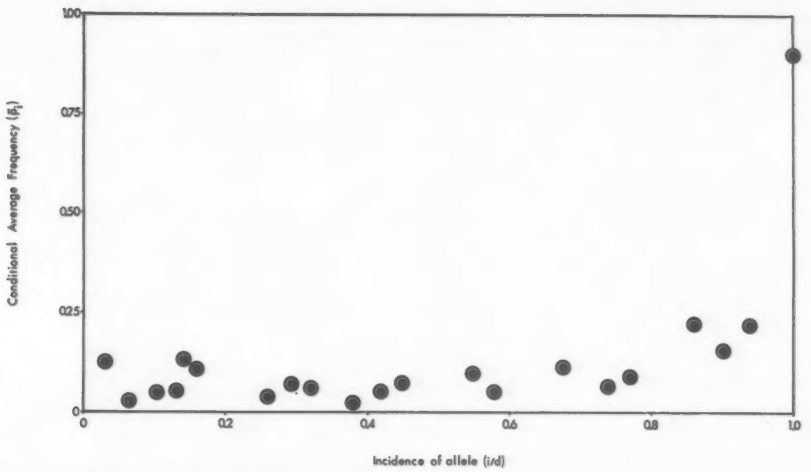

FIG. 3. Relationship between conditional average frequency of electrophoretic alleles, $\bar{p}_i$, and their incidence, i/d, in coastal rainbow trout populations.

TABLE 7. Sample allele frequencies of those alleles found to occur in only one ($i = 1$) or two ($i = 2$) populations of coastal rainbow trout. Site numbers refer to Fig. 1 and Table 1.

Locus (allele)	Frequency	Site
$i = 1$		
G3pdh-1 (85)	0.026	27
Icdh-1 (85)	0.450	4
Ldh-1 (150)	0.079	14
Me-2 (0)	0.032	10
Mdh-3,4 (110)	0.004	21
Gl-1 (80)	0.050	4
Gpi-1 (50)	0.417	14
Gpi-2 (66)	0.052	11
Gpi-3 (115)	0.196	31
Gpi-3 (60)	0.065	31
Sod (170)	0.009	27
$i = 2$		
Aat-1,2 (90)	0.005	26
	0.022	31
Icdh-3,4 (90)	0.005	26
	0.024	29
Ldh-3 (100)	0.032	26
	0.009	27
Ldh-3 (35)	0.011	26
	0.025	30
Phap (90)	0.032	2
	0.020	21
Gpi-1 (150)	0.017	4
	0.015	10

Although it seems intuitively reasonable that over sufficient time, populations that are geographically close would also be genetically more similar than geographically distant populations, this may not be entirely accurate when dealing with highly vagile species. With the exception of McIntyre (1976), most workers have found only weak correlations between genetic and geographic distances for salmonid populations (e.g. Kijima and Fujio 1982). We may hypothesize that geographic distance represents a porous barrier to gene flow by dispersal which is best described by a nonlinear function of both an organism's vagility and migratory precision. Unfortunately, attempts to model this have been unsuccessful. It is clear that clustered summary statistics (e.g. phenograms) offer only simplistic interpretations of reality (Felsenstein 1982).

The relative within-population gene diversity of 86.8% found in this study is close to values previously reported for rainbow trout (Allendorf and Phelps 1981a; Ryman 1983) and intermediate to those estimated for other salmonid species: chum salmon, 96.0–97.1%; pink salmon, 96.1%; sockeye salmon, 91.8–94.2%; coastal cutthroat trout, 91.0%; Atlantic salmon, 78.6%; and brown trout, 63.3% (Campton 1981; McGregor 1982; Okazaki 1982; Ryman 1983; Wilmot and Burger 1985). The low estimate of interpopulational gene diversity of 13.2% corroborates estimates of Nei's genetic identities in suggesting only moderate genetic divergence between populations.

Results of a gene diversity analysis between groups of populations above and below Shasta Dam indicated that only 1.6% of the total gene diversity may be ascribed to differences between regions. We may therefore hypothesize that either historical rates of gene flow between the regions were high and population sizes have remained relatively large or insufficient time has elapsed since the dam's closure in 1944 for the effects of genetic drift to become apparent. An additional explanation may be found in the suggestion that residual tetrasomy may substantially retard the effects of genetic drift in isoloci (Allendorf and Thorgaard 1984).

The recent development in graphical analysis of gene flow by Slatkin (1981) uses the frequency of individual alleles and their distribution among populations as indicators of gene flow. Results from the application of this method in conjunction with the locus by locus analysis using Wright's (1943) F-statistics indicated that $N_e m_e \geq 1.7$, where N_e is the effective population size and m_e is the effective migration rate. This value exceeds Spieth's (1974) "rule of thumb" that migration at the rate of one migrant per population per generation is sufficient to overcome the effects of genetic drift.

As expected from the high rate of gene flow, genetic variability at certain loci was expressed uniformly across all populations. Unexpectedly, we found that certain populations were distinguished by the presence of unique alleles occurring at

moderate frequencies. A similar case of a unique allele occurring at moderate frequency in a rainbow trout population has been reported (Allendorf and Phelps 1981a, Dickey River, WA, LDH-4 (50), $q = 0.240$). Within small, semiisolated populations, novel, effectively neutral mutations occur and may become common simply by genetic drift.

An explanation of the somewhat paradoxical situation of appreciable genetic differentiation among populations in spite of relatively high levels of within-population heterozygosity may be found in the speculations of Campton (1981) and the computer simulations of Altukov et al. (1984). Campton (1981) suggested that allele frequencies of individual salmonid populations, sampled at single points in time, are representative of essentially random samples of a greater global population. Individual populations are loosely tied together by gene flow which, over generational time, smooths out excessive departures from the panmictic allele frequencies. Modeling a single polymorphic locus, Altukov et al. (1984) has shown that under Kimura and Weiss' (1964) stepping stone model, allele frequencies predicted for partially isolated populations will fluctuate around panmictic means due to random effects of genetic drift and dampening effects of interpopulational gene flow. Globally stable states may indeed be maintained by differential fitnesses (Karlin 1981), but it is simply unnecessary to invoke selection as effector of the observed interpopulational gene diversity.

We therefore conclude that the 31 coastal rainbow trout populations studied were, until recently, part of a single, large genetic population. However, at any particular moment in time, local populations may appear to be randomly differentiating from each other at unlinked loci, while they are, in fact, merely expressing allele frequency fluctuations around a panmictic populational mean. The specific global mean around which allele frequencies fluctuate may be restricted by selective constraints with the amplitude of the fluctuations bound by gene flow.

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Effects of Diet on PCB-Induced Changes in Xenobiotic Metabolism in the Liver of Channel Catfish (*Ictalurus punctatus*)

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Effects of nutrition on xenobiotic-metabolizing enzymes in fishes are not well known. We fed three groups of channel catfish (*Ictalurus punctatus*) either a synthetic, laboratory-prepared diet or one of two commercial catfish rations. After 3 mo on each diet, fish were treated with the enzyme inducer Aroclor 1254. Hepatic microsomal monooxygenase (MO) activity was lower in untreated fish fed a commercial diet than in those fed the laboratory diet. Aroclor 1254 increased MO activity to a lesser degree in fish fed that same commercial diet compared with those receiving the laboratory or other commercial diet. Hepatic microsomal UDP-glucuronosyltransferase (UDPGT) activity was similar in all control fish; however, Aroclor 1254 increased UDPGT activity to differing degrees in the three diet groups. Hepatic cytosolic glutathione *S*-transferase (GST) activity was increased by Aroclor 1254 to a similar extent in all diet groups; however, levels of GST activity generally were higher in fish fed the commercial diets than in those fed the laboratory ration. These data indicate that diet can affect the toxicity of xenobiotics to fishes through alterations in hepatic enzyme systems, and thus should be considered a potential source of variation in toxicological studies with fishes.

On connaît mal l'incidence de l'alimentation sur les enzymes métabolisantes xénobiotiques présentes chez les poissons. Trois groupes de barbue de rivière (*Ictalurus punctatus*) ont été nourris d'un aliment expérimental synthétique ou d'une de deux rations commerciales à barbue. Après trois mois d'alimentation, on a incorporé l'inducteur d'enzymes Aroclor 1254 au régime. Le niveau d'activité hépatique de la monooxygénase microsomique (MO) était plus faible chez les poissons non traités nourris d'aliments commerciaux que chez ceux nourris d'aliments expérimentaux. On a noté une plus faible augmentation du niveau d'activité de la MO par suite de l'apport d'Aroclor 1254 chez les poissons nourris de ces mêmes aliments commerciaux par rapport à ceux nourris d'aliments expérimentaux ou d'autres aliments commerciaux. Le niveau d'activité hépathique de la UDP-glucuronosyltransférase (UDPGT) microsomique était semblable chez tous les poissons témoins; toutefois, l'Aroclor 1254 a entraîné une augmentation du niveau d'activité de la UDPGT, variable chez les trois groupes expérimentaux. L'effet stimulateur de l'Aroclor 1254 sur le niveau d'activité hépathique de la glutathion *S*-transférase (GST) cytisolique était semblable chez tous les groupes expérimentaux; toutefois, les niveaux d'activité de la GST étaient généralement plus élevés chez les poissons nourris d'aliments commerciaux que chez ceux nourris d'aliments expérimentaux. Les données révèlent que le régime alimentaire peut influer sur la toxicité des xénobiotiques pour les poissons par des modifications des systèmes enzymatiques hépathiques et devrait être donc considéré comme une source potentielle de variations dans les études toxicologiques menées sur des poissons.

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In mammals, numerous dietary factors influence toxicity of xenobiotics by affecting hepatic enzyme systems crucial in their metabolism. These systems include oxidative enzymes such as cytochrome P-450-dependent monooxygenases (MOs) and conjugating enzymes such as UDP-glucuronosyltransferases (UDPGTs) and glutathione *S*-transferases (GSTs). Dietary modifications that affect these enzymes in mammals include protein deficiency (Sachan 1975), lipid deficiency and/or type of lipid (Wade et al. 1985), deficiency of vitamin E, C, or A (Carpenter and Howard 1974; Sikic et al. 1977; Siddick et al. 1980), and certain naturally occurring or man-made compounds commonly found in foods (Wattenberg 1975; Benson et al. 1978; Sparnins et al. 1982).

Previous studies have shown that hepatic xenobiotic-metabolizing enzymes of fishes are functionally similar to those of mammals (Buhler and Rasmusson 1968; DeWaide 1971; James et al. 1979; Gregus et al. 1983). These enzymes in fishes also appear to be influenced by many of the same factors that affect

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mammalian enzyme systems (Binder et al. 1984). Relatively little is known, however, concerning the effects of nutrition on xenobiotic-metabolizing enzymes in fishes. Starvation of coho salmon (*Oncorhynchus kisutch*) or rainbow trout (*Salmo gairdneri*) for times ranging from 4 to 12 wk significantly decreased hepatic MO activity (Collodi et al. 1984; Andersson et al. 1985a). Stott and Sinnhuber (1978) reported that feeding rainbow trout different protein levels (32–62%) altered hepatic enzymes associated with xenobiotic metabolism. Other studies have shown that relatively high levels (≥50 μg/g) of cyclopropenoid fatty acids added to the diets of rainbow trout affect hepatic MO, epoxide hydrolase, and GST activities (Eisele et al. 1978, 1983). Most of the above studies, however, demonstrated effects only after rather radical dietary modifications. Virtually nothing is known concerning the influence of more subtle differences in diet on xenobiotic-metabolizing enzymes in fishes.

Slightly different diets can affect the toxicity of organic xenobiotics to fishes. Mehrle et al. (1974) reported that the acute toxicity of chlordane was significantly greater to rainbow trout fed several different commercial diets or a low-protein synthetic diet than to fish fed a high-protein synthetic diet. Similarly, rainbow trout and bluegill (*Lepomis macrochirus*) fed a synthetic diet were more tolerant of chronic exposure to Aroclor 1254 than fish fed a commercial diet (Mehrle et al. 1977). Other dietary factors that affect the sensitivity of fishes to organic xenobiotics include methionine and vitamin C (Mehrle et al. 1977; Mayer et al. 1978). Based on mammalian studies, Mehrle et al. (1977) suggested that nutritional effects on xenobiotic toxicity in fishes were related to alterations of enzymes involved in xenobiotic metabolism. From a toxicological standpoint, it is important to determine whether relatively minor differences in diet, such as those that occur among different commercial diets or between commercial and synthetic diets, can affect xenobiotic metabolism in fishes.

In this study, we examined hepatic MO, UDPGT, and GST activities in three groups of channel catfish (*Ictalurus punctatus*) fed either a synthetic laboratory diet or one of two commercial rations. Because hepatic MO, UDPGT, and GST activities in fishes can be increased by exposure to environmental contaminants such as polychlorinated biphenyls (PCB) (Andersson et al. 1985b; Ankley et al. 1986), we also examined the influence of diet on Aroclor 1254-induced changes in these enzyme systems.

Materials and Methods

Materials

Commercial rations were donated by Rangen, Inc., Buhl, Idaho, and Gold Kist, Inc., Atlanta, Georgia. Upon receipt, diets were frozen at −20°C to minimize nutrient loss. Ingredients for the synthetic diet were obtained from United States Biochemical Corporation, Cleveland, Ohio.[3] Sources for other reagents are described by Ankley et al. (1986).

Treatment of Fish

Fingerling channel catfish were obtained from Walton Fish Hatchery, Social Circle, Georgia, and maintained in a constant-flow system at the University of Georgia Whitehall Laboratory for approximately 5 mo. During this time the fish received a commercially available ration appropriate for their age. For the diet experiment, 300 uniformly sized fish (average weight 60.7 g) were divided randomly into three groups and placed in 850-L fiberglass tanks in a 90% reuse system. These fish were fed either (1) Goldkist Fish Food (0.625-cm sinking pellets), (2) Rangen Catfish Finisher (0.469-cm sinking pellets), or (3) a synthetic laboratory diet similar to that described by Durve and Lovell (1982) and prepared according to standard experimental guidelines (National Research Council 1983). Guaranteed minimum analyses of the Gold Kist and Rangen diets were, respectively, as follows: crude protein, 30.0 and 35.0%; crude fat, 3.0 and 5.0%; crude fiber, 8.0 and 10.0%. All essential vitamins were stated to be present in both commercial feeds, but actual levels were not indicated on the product labels. The composition of the laboratory diet is given in Table 1. All diets remained frozen until 1 d before use, when they were thawed at 4°C. Fish in each group were fed 2.0–2.5% of their body weight per day depending upon their size and the water temperature, which varied from 23 to 27°C. Fish were fed five to six times weekly (3 d on/1 d off). The fish generally consumed all food offered. In those instances when there was excess food, it was removed from the tanks within 10–20 min of feeding.

TABLE 1. Composition of synthetic diet.

Ingredient	Percentage
Vitamin-free casein	32.60
Gelatin	9.40
Dextrin	33.05
Cellulose	13.00
Corn Oil	4.00
Cod liver oil	3.00
Mineral mix[a]	4.00
Vitamin mix[b]	0.95

[a]Mineral mix of Williams and Briggs (1963) supplemented with cobalt chloride (1 mg/kg diet), aluminum potassium sulfate (0.7 mg/kg diet), and sodium selenite (0.08 mg/kg diet).

[b]Following given in mg/kg diet: ascorbic acid, 120; thiamin, 10; riboflavin, 20; pyridoxine, 10; folic acid, 5; Ca pantothenate, 40; choline chloride, 3000; niacin 150; vitamin B_{12}, 0.06; retinyl acetate (500 000 IU/g), 12; alpha tocopherol, 50; cholecalciferol (1 000 000 ICU/g), 6; menadione-Na-bisulfite, 80; inositol, 400; biotin, 2.

Fish were maintained on experimental diets for 90–92 d, during which no disease outbreaks were noted and no differential mortality occurred. Fish were starved for 1 d, and on days 91, 92, and 93, 40 fish were removed from the laboratory, Rangen, and Gold Kist tanks, respectively. Half were injected intraperitoneally with 2 mL corn oil/kg body wt (controls), and the other half were treated with Aroclor 1254 dissolved in an equal volume of corn oil (50 mg Aroclor 1254/kg body wt). After treatment, the fish were placed in separate 300-L fiberglass tanks in a constant flow of 20°C water. Fish were not fed after being treated and were sampled 4 d later. To ensure consistency of treatment and sampling, the same schedules, equipment, reagents, and personnel were used for all procedures.

Tissue Preparation and Enzyme Assays

Eighteen fish from each diet/treatment group were killed by decapitation and their livers were pooled randomly in groups of two. Livers were homogenized in four volumes of a cytochrome P-450-stabilizing buffer (0.1 M KH_2PO_4 at pH 7.25, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol), and microsomes and cytosol were prepared by differential centrifugation

[3]Mention of trade names or commercial products does not constitute endorsement or recommendation by the U.S. Fish and Wildlife Service.

as described previously (Ankley et al. 1986). Tissue fractions were stored at −80°C until analyzed. Microsomal enzymes were assayed within 1 wk of preparation, and GST activities were measured within 3 wk.

The *O*-dealkylation of 7-ethoxyresorufin (EROD) and 7-ethoxycoumarin (ECOD) by microsomal MOs was measured during fluorimetric procedures reported by Burke and Mayer (1974) and Ullrich and Weber (1972), respectively, as described previously (Ankley et al. 1986). Microsomal UDPGT activity was assayed using 4-methylumbelliferone as the aglycone substrate with 0.1% Triton X-100 in the incubation mixtures (Ankley et al. 1986; Ankley and Agosin 1987). GST activities toward 1-chloro-2,4-dinitrobenzene (CDNB), *p*-nitrobenzyl chloride (NBC), 1,2-epoxy-3-(*p*-nitrophenoxy)propane (ENPP), and ethacrynic acid (EA) were determined using the spectrophotometric procedures detailed by Habig et al. (1974). Microsomal enzymes were assayed at 30°C, and GST activities were determined at 20°C. Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Diet Analyses

After the feeding experiment had been completed, several components of the commercial diets were analyzed by Woodson-Tenent Laboratories, Inc., Gainesville, Georgia.

Statistical Procedures

Differences in body weight among the diet groups were tested using one-way analysis of variance, followed by Duncan's multiple range test. The rest of the data were analyzed using two-way analysis of variance with diet and treatment as the main factors. Interaction between diet and treatment also was tested. When analysis of variance indicated that diet and/or treatment effects were significant, but interaction was not, mean separations were performed using Duncan's multiple range test. When diet × treatment interaction was significant, various pairwise comparisons were made using Student's *t*-test. SAS 82 (SAS Institute Inc. 1982) was used for all statistical analyses, and results were considered significant at $p \leq 0.05$.

Results

Growth varied significantly among the diet groups, with the best growth occurring in fish receiving the Rangen diet, followed by those fed the laboratory diet and then those receiving the Gold Kist ration (Table 2). Although there were differences in growth rates among the diet groups, all fish were healthy and none exhibited gross lesions or deformities.

Hepatic microsomal EROD, ECOD and UDPGT activities all were significantly affected by both diet and treatment; however, there was a significant diet × treatment interaction with each of these three variables (Table 3).

Treatment with Aroclor 1254 increased EROD activity 4.4-, 4.7-, and 3.4-fold, relative to controls, in fish fed the laboratory, Rangen, and Gold Kist diets, respectively (Table 3). The diet × treatment interaction probably was due to the relatively lesser increase in EROD activity in the Aroclor-treated fish fed the Gold Kist diet. Comparison of controls fed the laboratory diet with those fed the Gold Kist ration, using Student's *t*-test, indicated that the latter had significantly lower levels of EROD activity ($p < 0.01$). Also, Aroclor 1254-treated fish fed the laboratory and Rangen diets had significantly higher levels of EROD activity than the Aroclor-treated fish fed the Gold Kist diet ($p < 0.001$ and $p < 0.005$, respectively).

TABLE 2. Final body weights of channel catfish fed three different diets. Data are $\bar{X} \pm$ SD for 40 fish. The three values differed significantly ($p \leq 0.05$) from one another. The mean initial weight of the fish was 60.7 g.

	Diet		
	Laboratory	Rangen	Gold Kist
Body wt (g)	182.0 ± 33.3	198.5 ± 37.2	153.0 ± 31.4

Hepatic microsomal ECOD activity followed the same qualitative pattern as EROD activity (Table 3). Within diet group comparison of ECOD activity in control versus Aroclor-treated fish indicated that the 85 and 70% increases in activity in fish fed the laboratory and Rangen rations were significant ($p < 0.0005$ and $p < 0.0001$, respectively) whereas the 20% increase in the Gold Kist group was not. Thus, it appears that the diet × treatment interaction was due to the relatively small increase in ECOD activity in the Aroclor-treated fish fed the Gold Kist diet. Comparison of levels of ECOD activity in the controls indicated that there were no significant differences among the diet groups; however, ECOD activity in the Aroclor-treated fish was significantly higher in the fish fed the laboratory and Rangen diets than in those receiving the Gold Kist ration ($p < 0.0001$ and $p < 0.0001$, respectively).

Treatment with Aroclor 1254 significantly increased hepatic microsomal UDPGT activity by about 35 and 80%, relative to controls, in fish fed the laboratory and Rangen diets ($p < 0.005$ and $p < 0.0005$, respectively) (Table 3). However, UDPGT activity was not increased in Aroclor-treated fish fed the Gold Kist diet (Table 3). Thus, the diet × treatment interaction again would seem to be, at least in part, due to the lack of increase in UDPGT activity in Aroclor-treated fish from the Gold Kist group. There were no significant differences among the diet groups in control levels of UDPGT activity; however, activity in the Aroclor-treated fish was significantly higher in the laboratory and Rangen groups than in fish fed the Gold Kist ration ($p < 0.05$ and $p < 0.0001$, respectively). Also, UDPGT activity in Aroclor-treated fish fed the Rangen diet was significantly higher than in those fed the laboratory ration ($p < 0.01$).

Hepatic cytosolic GST activity exhibited markedly different trends than did the activities of the microsomal enzymes (Table 4). GST activity in fish fed the two commercial diets generally was higher than GST activity in fish fed the laboratory ration. Also, although GST activity toward four different substrates was significantly affected by diet and/or treatment, there was no significant diet × treatment interaction.

GST activity toward CDNB was influenced by both diet and treatment (Table 4). Activity toward this substrate was increased 10–13% in the Aroclor-treated fish and was significantly higher in the Gold Kist and Rangen groups than in fish fed the laboratory diet. GST activity toward NBC also was affected by diet, but not by treatment with Aroclor 1254 (Table 4). Activity toward NBC was significantly higher in fish fed the Gold Kist diet than in those fed either the Rangen or laboratory rations. When ENPP was used as a GST substrate, diet did not influence activity; however, treatment with Aroclor 1254 significantly increased activity to varying degrees in the three diet groups (Table 4). Activity toward ENPP was increased approximately 22, 8, and 1% in the Aroclor-treated fish fed the laboratory, Rangen, and Gold Kist diets, respectively. Although GST activity toward this substrate did not appear to be increased in

treated fish fed the Gold Kist diet, the analysis of variance did not indicate a significant diet × treatment interaction. GST activity with EA as a substrate was affected by both diet and treatment (Table 4). Levels of activity toward EA were increased 8–20% in fish treated with Aroclor 1254, and activity was significantly higher in fish fed the Gold Kist diet than in those receiving either the laboratory or Rangen rations.

After the diet studies were completed, the two commercial feeds were analyzed for several components (Table 5). Levels of crude protein were very close to those listed on the product labels (Materials and Methods). In both diets, total fat was somewhat higher than that listed in the guaranteed analyses (Table 5). Levels of vitamin A and vitamin E were similar in both diets and are within the range considered adequate for catfish (Table 5) (National Research Council 1983). The level of vitamin C in the Rangen diet (43.6 mg vitamin C/kg diet) was slightly lower than the 60 mg/kg suggested for catfish (Durve and Lovell 1982), and the level of vitamin C in the Gold Kist diet was extremely low (<4.4 mg/kg). There was no detectable peroxidation of the Gold Kist feed, and the Rangen diet had a peroxide value of 9.6 meq/kg, which is well within acceptable limits (Hung and Slinger 1980).

Fatty acid compositions of the commercial and laboratory diets are reported elsewhere (Ankley 1987). There were marked quantitative differences in levels of fatty acids between the Rangen and Gold Kist diets (Table 5); however, qualitative differences were relatively minor. In most cases, levels of fatty acids in the laboratory diet were similar to those in the Rangen ration (Ankley 1987). The laboratory diet, however, had some types of fatty acids not found in either commercial diet. These were primarily long-chained, polyunsaturated fatty acids that were components of the cod liver oil.

Discussion

Very little is known concerning possible effects of nutrition on xenobiotic metabolism in fishes. We found significant differences in hepatic xenobiotic-metabolizing enzymes among three groups of ostensibly healthy channel catfish fed two different commercial rations or a synthetic diet for 3 mo. To our knowledge, this is the first report of variations in the activities of xenobiotic-metabolizing enzyme systems of fishes fed different commercial or commercial versus synthetic diets. Not only did control levels of MO and GST activities vary among the diet groups, but Aroclor 1254-induced changes in MO and UDPGT activities also were dependent upon diet. In controls, MO and GST activities in fish fed the Gold Kist diet were significantly lower and higher, respectively, than corresponding activities in fish receiving the laboratory ration. Controls from the Rangen group generally exhibited MO and GST activities that were intermediate to the laboratory and Gold Kist groups. The effects of Aroclor 1254 on GST activity in the different diet groups were quite similar; however, diet significantly influenced the responses of the microsomal enzymes to this PCB mixture. Generally, MO and UDPGT activities in fish fed the laboratory and Rangen diets were increased similarly and to a relatively greater degree than fish receiving the Gold Kist ration.

The observed variations in GST and, in particular, MO activities among the three diet groups were of sufficient magnitude to suggest that there would be differences in the sensitivity of these fish to certain types of organic xenobiotics. These data agree with previous studies that found fishes fed different diets differ in their sensitivity to acute or chronic exposure to organochlorine compounds (Mehrle et al. 1974, 1977; Mayer et al. 1978). Marking et al. (1984) found that diet may not always be an important factor in determining the acute toxicity of xenobiotics to fish; however, the rainbow trout used in their experiments were quite small (≤1 g), relative to the catfish used in our study. Thus, size or age of fishes may have a significant bearing on the relative importance of nutritional effects on xenobiotic toxicity. Our results suggest that nutrition can be a significant factor contributing to variability in toxicological data generated in different laboratories for a given fish species.

Because the compositions of the diets used in this study were somewhat different, it is not possible to make conclusive statements concerning mechanisms underlying the observed differences in enzyme activities among the diet groups. It is possible, however, to form some plausible hypotheses. Hepatic MO activities are decreased significantly in vitamin C-deficient guinea pigs (Wagstaff and Street 1971; Zannoni et al. 1972; Sikic et al. 1977). Thus, it is possible that the significantly lower MO activity in fish fed the Gold Kist diet resulted from a vitamin C deficiency. Mayer et al. (1978) reported that channel catfish fed 670 or 5000 mg vitamin C/kg diet had higher hepatic MO activity than those fed 63 mg/kg. This latter level of vitamin C is considered nutritionally adequate for catfish and is higher than levels of vitamin C present in either of the commercial diets in our study. Therefore, based on the results of Mayer et al. (1978), it may be conjectured that overall trends in MO activity among the diet groups in the present study were related to dietary levels of vitamin C. The highest MO activity was in fish fed the laboratory diet (120 mg vitamin C/kg), followed by the Rangen diet (43.6 mg/kg) and then the Gold Kist diet (<4.4 mg/kg).

It is more difficult to hypothesize causative factors for the relative lack of increase in hepatic MO and UDPGT activities in Aroclor-treated fish fed the Gold Kist diet. Based on mammalian studies, these lesser increases in enzyme activity could

TABLE 3. Effects of diet and treatment with corn oil (control) or Aroclor 1254 on two MO activities and UDPGT activity in channel catfish. Data are expressed as $\bar{X} \pm$ SD for nine groups of two pooled livers. MO activities are given as pmol resorufin (EROD) or 7-hydroxycoumarin (ECOD) formed·min^{-1}·mg microsomal protein^{-1} and UDPGT activity is given as nmol 4-methylumbelliferone conjugated·min^{-1}·mg microsomal protein^{-1}. Effects due to diet are significant at $p < 0.0005$, effects due to treatment are significant at $p < 0.0001$, and the diet × treatment interactions are significant at $p < 0.005$.

	EROD		ECOD		UDPGT	
Diet	Control	Aroclor 1254	Control	Aroclor 1254	Control	Aroclor 1254
Laboratory	19.5±8.7	86.3±21.4	11.3±3.6	20.9±5.3	1.17±0.16	1.59±0.29
Rangen	15.3±6.4	72.4±22.8	10.4±2.5	17.6±2.6	1.24±0.43	2.21±0.50
Gold Kist	10.6±2.7	36.4±18.8	8.6±1.8	10.3±2.9	1.23±0.39	1.19±0.29

TABLE 4. Effects of diet and treatment with corn oil (control) or Aroclor 1254 on cytosolic GST activity toward four substrates in channel catfish. Data are expressed as $\bar{X} \pm$ SD for nine groups of two pooled livers, and activities are given as nmol substrate conjugated· min^{-1}· mg cytosolic $protein^{-1}$.

	1-Chloro-2,4-dinitrobenzene[a,b,c]		*p*-Nitrobenzyl chloride[a,d]		1,2-Epoxy-3(*p*-nitrophenoxy) propane[b]		Ethacrynic Acid[a,b,d]	
Diet	Control	Aroclor 1254	Control	Aroclor 1254	Control	Aroclor 1254	Control	Aroclor 1254
Laboratory	741.5±59.5	813.8±120.7	57.3±5.6	63.6±8.1	25.0±3.4	30.6±5.0	17.1±2.5	20.0±2.2
Rangen	811.0±111.2	890.0±110.0	61.4±9.2	63.9±6.2	28.2±5.8	30.5±3.5	16.9±2.5	18.3±1.7
Gold Kist	841.7±69.0	954.2±78.4	71.3±10.2	72.6±11.3	30.0±5.2	30.2±5.9	19.4±2.1	23.0±3.8

[a]Effects due to diet are significant at $p < 0.005$.
[b]Effects due to treatment are significant at $p < 0.05$.
[c]Comparison of diets (summed over treatment) with Duncan's multiple range tests revealed Gold Kist = Rangen > laboratory ($p \leq 0.05$).
[d]Comparison of diets (summed over treatment) with Duncan's multiple range test revealed Gold Kist > Rangen = laboratory ($p \leq 0.05$).

TABLE 5. Levels of crude protein, fat, vitamin A, vitamin E, and vitamin C, and peroxide values for the commercial diets. Analyses were done by Woodson-Tenent Laboratories, Inc., Gainesville, Georgia.

Component	Diet	
	Rangen	Gold Kist
Crude protein (% of total diet)	34.76	29.95
Fat (% of total diet)	8.88	4.08
Vitamin A (IU/kg diet)	7040.00	8360.00
Vitamin E (IU/kg diet)	173.80	143.30
Vitamin C (mg/kg diet)	43.60	<4.40
Peroxide value (meg/kg diet)	9.60	0.00

be due to a vitamin C deficiency (Wagstaff and Street 1971), or they may be related to the low fat content of the Gold Kist diet, compared with the Rangen or laboratory rations (Wade et al. 1985). Also, an interactive effect between low vitamin C and low fat levels in the Gold Kist group cannot be ruled out.

Another factor that possibly could have influenced MO and/or UDPGT activities in fish fed the different diets was the quality of protein. Although the quantity of protein was similar among dietary groups, the quality (availability and/or amino acid composition) could have been quite different. In mammals, protein quality has been shown to influence xenobiotic-metabolizing enzymes, and thus xenobiotic toxicity (Campbell and Hayes 1974).

Hepatic cytosolic GST activity in fish fed the commercial diets was consistently higher than in those receiving the laboratory ration. This effect was especially apparent in the Gold Kist group. Studies with guinea pigs have shown that vitamin C deficiency does not affect, or slightly decreases, hepatic GST activity (Sikic et al. 1977; Zannoni et al. 1982). Therefore, some other dietary factor might be involved in the higher GST activity observed in the fish fed the commercial diets. It is known that several naturally occurring or man-made compounds commonly found in commercial feeds can increase GST activity in mammals (Benson et al. 1978; Sparnins et al. 1982). Further research is required, however, to determine whether levels of these types of compounds present in commercial fish feeds are sufficient to cause increased GST activity.

In fish fed the laboratory and Rangen diets, the effects of Aroclor 1254 on MO and UDPGT activities were quite similar to those we observed in other studies with this PCB mixture (Ankley et al. 1986, 1987). However, there are some differences between the present study and previous work with respect to the effects of Aroclor 1254 on GST activity. Previously, we found that treatment of catfish with this PCB mixture significantly increased GST activity toward ENPP, but not toward CDNB, NBC, or EA (Ankley et al. 1986). In the present study, exposure to Aroclor 1254 increased GST activity toward CDNB and EA, as well as ENPP. The explanation for this observation is not readily apparent; however, given the relatively small increases in GST activity observed in Aroclor-treated catfish (8–20%), perhaps it is not surprising that results between studies are variable.

In summary, our results indicate that diet can be a significant factor influencing the enzymatic metabolism, and thereby toxicity, of organic xenobiotics in fishes. It would be interesting to compare the toxicological responses of fish from the different diet groups in our study to various xenobiotics. Fish fed the Gold Kist diet quite possibly would be more sensitive than those fed the laboratory or Rangen diets to xenobiotics, such as organochlorines, that are detoxified by MOs. With other types of xenobiotics, however, fish fed the Gold Kist diet may be more tolerant than those receiving the laboratory or Rangen rations. For example, certain polycyclic aromatic hydrocarbons (PAH) are activated by MOs to carcinogenic metabolites which in turn are detoxified by conjugating enzymes, such as GSTs (Jefcoate 1983). Thus, PAH may be less carcinogenic in animals, such as fish fed the Gold Kist diet, with low MO and high GST activities. Therefore, one cannot make all-encompassing statements concerning the relative benefits of one diet versus another, with respect only to differences in xenobiotic toxicity. Overall, our results suggest that to reduce variability in organic xenobiotic toxicity data for fishes it may be necessary to use standardized diets, which is a routine procedure in mammalian studies.

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An In Situ Experimental Evaluation of the Elliott and Persson and the Eggers Models for Estimating Fish Daily Ration[1]

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Boisclair, D., and W. C. Leggett. 1988. An in situ experimental evaluation of the Elliott and Persson and the Eggers models for estimating fish daily ration. Can. J. Fish Aquat. Sci. 45: 138–145.

We compared estimates of daily ration developed using the theoretically rigorous and logistically demanding Elliott and Persson model and the more easily applied Eggers model which is infrequently used because of its assumptions about rigid fish feeding periodicity. Comparisons were based on ten 24-h samplings of six different yellow perch (*Perca flavescens*) populations. Daily ration estimates from the two models did not differ significantly. This consistency occurred in spite of the fact that in some cases the observed feeding periodicity violated the assumptions of the Eggers model. A simulation model demonstrated that 95% confidence intervals were smallest for the Eggers estimates and that the Eggers model was more robust than the Elliott and Persson model to changes in both sampling frequency and number of fish sacrificed at each sampling event. The latter proved particularly sensitive to changes in sampling frequency. We concluded that the two models provide estimates of daily ration comparable in magnitude and accuracy and consequently that the restriction of the Eggers model to fish with rigid feeding periodicity is not justified. Furthermore, the Eggers model, because of its robustness, reduces the sampling requirements to determine daily ration, and hence, permits its estimation on a more frequent basis.

Nous avons comparé des estimés de ration journalière développés à l'aide du modèle théoriquement rigoureux mais pratiquement exigeant de Elliott et Persson et du modèle de Eggers qui, bien que plus facile à appliquer, n'est pas fréquemment utilisé à cause de prémisses concernant la rigidité de la périodicité de la nutrition. Les comparaisons furent basées sur dix échantillonnages de 24 h de six différentes populations de perchaudes (*Perca flavescens*). Les estimés de ration journalière des deux modèles ne furent pas significativement différents malgré le fait que dans certains cas, la périodicité de nutrition outrepasse les prémisses du modèle de Eggers. Une simulation démontra que les intervalles de confiance à 95 % étaient plus petits pour les estimés de Eggers et que le modèle de Eggers était plus robuste aux changements de fréquence et à la taille de l'échantillonnage que celui du modèle de Elliott et Persson. Ce dernier modèle fut particulièrement sensible aux changements de la fréquence d'échantillonnage. Nous concluons que les deux modèles donnent des estimés de ration journalière comparables en terme de magnitude et de précision et conséquemment que la restriction du modèle de Eggers à des poissons à périodicité rigide de nutrition n'est pas justifiée. De plus, le modèle de Eggers, à cause de sa robustesse, réduit l'effort d'échantillonnage pour déterminer la ration journalière, et ainsi, permet son estimation sur une base routinière.

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The quantity of of food consumed by fish is typically estimated on a daily basis. It requires a survey of the quantity of food found in the stomachs of individual fish during the course of one day and an estimate of the evacuation rate prevailing during the day.

The choice of a model to estimate daily ration is dictated by the form of the gastric evacuation function. Some models assume a linear gastric evacuation (Bajkov 1935; Hunt 1960; Seaburg and Moyle 1964; Daan 1973); others use a square root (Jobling 1981) or exponential model (Eggers 1977; Clarke 1978; Elliott and Persson 1978). Some daily ration models accommodate a complete range of assumptions about the pattern of gastric evacuation (Pennington 1985; Olson and Mullen 1986). Despite the variety of gastric evacuation models available, the exponential model is most commonly used (Tyler 1970; Brett and Higgs 1970; Elliott 1972). It has been argued that the exponential model generally gives a better description of the rate of food evacuation than alternative models (Persson 1986). This is especially true when fish are fed on relatively small and low-energy prey (Jobling 1986).

Of the daily ration models that assume an exponential gastric evacuation model, the Elliott and Persson (1978) model is widely accepted as the most theoretically rigorous (Cochran 1979; Eggers 1979; Elliott 1979). This model requires that estimates of the quantity of food in the stomach be obtained at approximately 3-h intervals and than an estimate of the evacuation rate during that interval also be obtained. These are combined to sequentially evaluate the quantity of food consumed between each sampling time. Daily ration is determined by

[1]Contribution of the Limnology Research Center, McGill University.

summation, over 24 h, of the several estimates of food consumption per 3-h period.

The Elliott and Persson model has the advantage of being applicable to almost any temporal feeding pattern, but it does have two important limitations: (1) it is logistically demanding, since fish must be collected at approximately 3-h intervals and should be dissected immediately following their capture to avoid bias due to *post mortem* evacuation and regurgitation, and (2) it is an algebraically complex model which involves the repetitive use of several parameters (especially evacuation rate) during computation. Consequently, it suffers the statistical disadvantage of increasing the standard errors around the estimates. A logistically less demanding method, which yields equivalent results, is badly needed.

The model of Eggers (1977), generally perceived as a special case of the Elliott and Persson model, meets some of these needs. The Eggers model computes daily ration as the product of the average quantity of food found in the digestive tract over 24 h and the evacuation rate.

The main limitation of the Eggers model is that it is believed to be applicable only when feeding periodicity is such that the quantities of food in the digestive tract at the beginning and the end of a 24-h period are equal (Elliott 1979; Pennington 1985). Use of the Eggers model has thus been restricted to fish that exhibit very rigid feeding patterns. The magnitude of errors introduced by relaxing these theoretical limits on feeding patterns has never been explored. An important advantage of this model is its use of the daily mean quantity of the food in the digestive tract, which makes an increased sampling interval possible. Sampling effort is thus reduced. Its computational simplicity should also increase the statistical precision of daily ration estimates.

In this study we compared estimates of daily ration developed from the same data sets using both the Elliott and Persson (1978) and the Eggers (1977) models. In particular we examined the effects of variable feeding periodicities and different sampling frequencies on the estimates produced by the two models.

Methodology

Study Area

Ten separate estimates of daily ration were developed for six yellow perch (*Perca flavescens*) populations located in the Eastern Townships of Quebec (Fig. 1). Perch were studied because they are abundant in the study area. We estimated daily ration for perch ranging in size between 50 and 150 mm total length (TL) in Lakes Memphremagog, Hertel, Magog, D'Argent, Waterloo, and Brome and between 150 and 250 mm TL in Lakes Memphremagog, Magog, Waterloo, and Brome (Table 1). During each sampling session, we determined diel variability in digestive tract contents and completed an experiment to determine evacuation rate. In this paper, each of the 10 sampling sessions will be referred to as a "trial." Data collected during each trial were used to develop daily ration estimates using both the Eggers (1977) and the Elliott and Persson (1978) models.

Diel Variability in Digestive Tract Content

For each trial, daily variation in the content of the complete digestive tract (stomach plus intestine) was evaluated by collecting perch with a 50 × 3 m knotless nylon bag seine (0.6-cm² mesh). A total of seven seine hauls was executed at 4-h intervals over a 24-h period. An equal number of fish (n) was retained at each 4-h interval. The sample size n varied from 6 to 20 between trials (Table 1). The fish sampled were killed by an overdose (1 mL·L^{-1}) of 2-phenoxyethanol immediately following capture. Digestive tract contents were removed within 2 h of capture and fish fresh weight (excluding digestive tract contents) were determined (±0.01 g wet wt) on site with an electronic balance. The unpreserved digestive tract contents

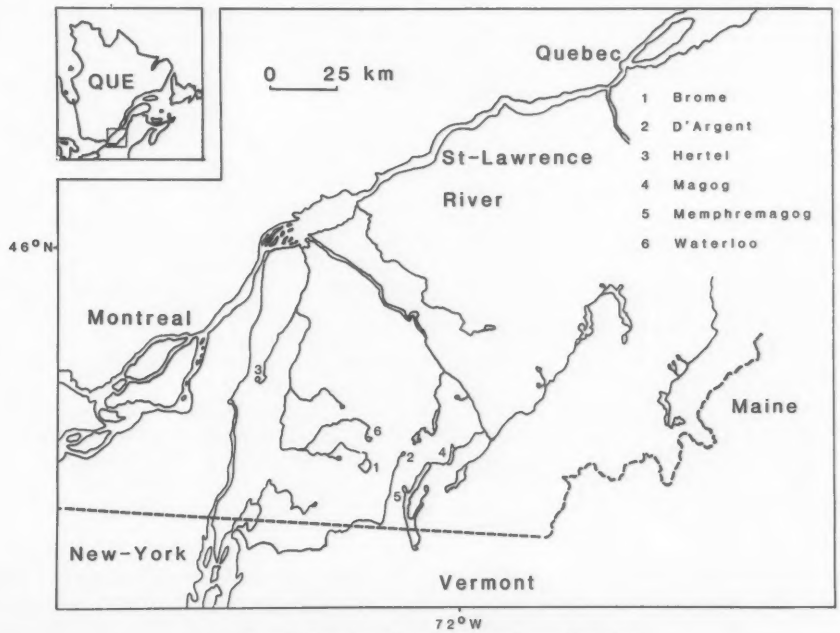

FIG. 1. Location of study area and sampling sites.

TABLE 1. Sampling protocol for estimates of daily ration.

Trial	Date	Lake	Size class mm(TL)	Number of fish collected per 4-h interval (n)
1	23/05/84	Memphremagog	50–150	12
2	23/05/84	Memphremagog	150–250	6
3	08/06/84	Hertel	50–150	20
4	06/07/84	Magog	50–150	6
5	06/07/84	Magog	150–250	15
6	19/07/84	D'Argent	50–150	10
7	26/07/84	Waterloo	50–150	20
8	26/07/84	Waterloo	150–250	14
9	02/08/84	Brome	50–150	20
10	02/08/84	Brome	150–250	10

from each fish were placed in separate 5-cm aluminum weighing pans and immediately dried on site to constant weight 60°C (48 h).

The gut fullness, F_t, (grams dry weight per 100 g wet weight), of each fish sampled at time t was determined using equation 1:

$$(1)\quad F_t = \frac{G_t}{W_t} \times 100$$

where G_t is the dry weight (grams) of the digestive tract contents and W_t is the total wet body weight (grams).

Evacuation Rate (Field)

Evacuation rate was estimated on site during each trial. Fifty to 100 perch were collected by seining at the beginning of the sampling interval for that trial. These were immediately stocked into a portable 1-m-diameter circular pool located at lake side adjacent to the sampling site. A continuous flow (500 $L \cdot h^{-1}$) of filtered (75 μm) lake water was supplied to the pool by a battery powered electric bilge pump. The pump intake was positioned 20 m from the shoreline at a depth of approximately 2 m. Water temperatures in the pool were always within ±1° of the mean epilimnion water temperature.

Ten to 20 perch were sacrificed and processed as previously described at the time of stocking and at each successive 4- to 5-h interval for 20–24 h. Gut fullness (F_t) for each fish collected was estimated at time of sacrifice using equation 1.

Several authors (Kitchell et al. 1978; Nakashima 1979; Grove and Crawford 1980; Persson 1982) have shown that the decrease in the gut fullness of unfed fish can be accurately described by the exponential model

$$(2)\quad \bar{F}_{(t+1)} = \bar{F}_t\, e^{-RT}$$

which, in its logarithmic form, is

$$(3)\quad \ln \bar{F}_{(t+1)} = \ln \bar{F}_t - RT$$

where $\bar{F}_t$ and $\bar{F}_{(t+1)}$ are, respectively, the mean gut fullness at the beginning and end of time interval T (4–5 h). The evacuation rate, R, for each trial was estimated as the slope of the relationship between $\ln \bar{F}_t$ and time.

Evacuation Rate (Laboratory)

Although field evacuation experiments represent realistic expressions of fish size, diet and feeding schedule, and water temperature experienced by fish at liberty, they involve recently caught fish that may have been stressed due to capture and handling. If stress occurred, it could depress estimates of R (Mann 1967; Swenson and Smith 1973; Thorpe 1977; Windell 1978). To evaluate this possibility, we conducted seven experiments in the laboratory. Three experiments used perch from Lake Hertel, two from Lake Magog, and two from Lake Memphremagog.

For each experiment, 50–100 perch collected by seining were transported to McGill University in a portable 0.75-m^3 tank filled with lake water that was cooled with ice packs and aerated by air stones connected to a SCUBA tank. Transport time varied between 1 and 3 h. Mortality during capture and transport was <5%. At McGill, perch were placed in "Living Stream" temperature-regulated stream tanks. All fish were fed to satiation with brine shrimp (Anostraca: Artemia) twice daily to simulate their natural feeding rhythm (Boisclair and Leggett 1985). Voluntary feeding normally resumed within 1 d of transfer. A small posttransfer mortality was common. No mortality occurred beyond 5 d posttransfer, and the maximum mortality during capture, transfer, and holding was 10%. Subsequent to transfer, water temperature was altered by 1° per day until the desired experimental water temperature was attained. The fish were then maintained at that temperature for at least 1 wk before beginning an evacuation experiment.

Each evacuation experiment was initiated 5–6 h after the fish were last fed to insure that no food was left in the tanks at the beginning of the experiment. The protocols for fish sampling and for computations were identical to those employed in the field experiments.

We believe that the laboratory experiments provided reliable nonstress values because (1) the fish were given 1–3 wk to adapt to their new environment and (2) the brine shrimp diet fed to perch in the laboratory experiments closely approximated the amphipods (Amphipoda: Hyalella) and other crustaceans that composed an important part of the diet of wild fish (average 35% by weight; D. Boisclair and W. C. Leggett, in prep). Amphipods and brine shrimp are not only closely related taxonomically but are morphologically and energetically similar (4878 and 5834 Cal·g dry wt^{-1}, respectively; Cummins and Wuycheck 1971).

We adjusted the field evacuation rate estimates on the assumption that handling stress had depressed the field derived estimates of R. To do so we used the following equation:

$$(4)\quad \ln R' = \ln R \times \frac{\ln \hat{R}_1}{\ln \hat{R}'}$$

where R and R' are, respectively, the uncorrected and corrected field-derived evacuation rates and $\hat{R}$ and $\hat{R}_1$ are the evacuation rates predicted for a given temperature by equation 9 (in situ rates) and 10 (experimental rates), respectively (see Results). Each R' was assigned a standard error equal to that prevailing before the correction.

To evaluate the hypothesis that the difference between field- and laboratory-derived estimates of R was due to food type differences, we tested the postulate that the factor by which field evacuation rates were corrected should be lower for trials that had a diet closer to that of fish in the laboratory (i.e. high percentage of amphipods in the diet).

Computation of Point Estimates of Daily Ration

Mean daily ration, $\bar{D}$, was estimated for each of the 10 trials using both the Eggers (1977) and the Elliott and Persson (1978) models.

The Eggers model defines daily ration as

(5) $\bar{D} = \bar{F} \times R' \times 24$

where $\bar{F}$ is the mean gut fullness of all fish collected during a trial, R' is the corrected evacuation rate prevailing during that day, and 24 is the number of hours per day.

The Elliott and Persson model defines daily ration as

(6) $\bar{D} = \sum_{\Delta t=1}^{p} \bar{C}_{\Delta t}$

where $\bar{C}_{\Delta t}$ is the quantity of food consumed by perch during an interval Δt between two successive sampling periods t and $t + 1$ and p is the number of intervals per day. In our study there were always six such intervals. $\bar{C}_{\Delta t}$ is defined as

(7) $\bar{C}_{\Delta t} = \dfrac{(\bar{F}_{(t+1)} - \bar{F}_t e^{-R'T}) R'T}{(1 - e^{-R'T})}$

where $\bar{F}_t$ and $\bar{F}_{(t+1)}$ are the mean gut fullness at two successive sampling periods, R' is the (corrected) evacuation rate for that trial, and T is the time between two successive sampling periods (4 h in our study). Mean gut fullness was estimated as Arcsin $\sqrt{F_t}/100$ and was employed in equations 5 and 7 in its back-transformed form.

Computation of the Error Around Daily Ration Estimates

To assess the error around the values provided by equations 5 (Eggers) and 6 (Elliott and Persson), we used a simulation technique derived from the bootstrap method (Efron 1981). A simulation was run independently for each trial. Following this method, equations 5 and 6 were computed with individual values of F_t drawn at random from the data of a trial and combined with pseudovalues R^* obtained by equation 8:

(8) $R^* = R' + (h \times s_{R'})$

where R' is the corrected evacuation rate, $s_{R'}$ is its standard error, and h is random variate generated from a distribution having a mean of 0 and a standard deviation of 1.

For each trial, two sets of daily ration estimates were thus derived: one set of $7 \times n$ estimates based on the Eggers model and one set of n estimates based on the Elliott and Persson model (Table 1). These sets were then used to test the significance of differences between daily ration estimates (Arcsin $\sqrt{D}/100$ transformed data) both between models on a given trial and between trials.

Effect of Sampling Frequency and Number of Fish Sampled

To assess the effect of the sampling size and frequency on daily ration estimates, we simulated the results of both models while varying the number of fish collected per sampling period from 5, 10, 15, to 20 and the sampling interval from 4, 8, to 12 h.

Results

Diel Variability in Digestive Tract Content

Mean gut fullness ranged from 0.21 for Trial 8 to 0.69 for Trail 3 (Fig. 2). The quantity of food in the digestive tracts of perch varied significantly both between trials ($F_{9,861} = 30.29$; $p < 0.0001$) and with time of day ($F_{6,861} = 18.45$; $p < 0.0001$). The significance of the interaction term ($F_{54,861} = 2.52$; $p < 0.0001$) indicated a lack of synchrony in the diel variability of gut fullness between trials. Differences in gut fullness during the 24-h sampling period were not significant for Trials 1–3 ($p > 0.20$), but were highly significant for Trials 4–10 ($p < 0.005$). Gut fullness at the beginning and the end of the 24-h period were significantly different at $p < 0.08$ for Trial 4, $p < 0.03$ for Trial 5, and $p < 0.02$ for Trial 9 (least square means a posteriori test; Sokal and Rohlf 1981). The ratio of gut fullness at the beginning and the end of the 24-h period was 2 for Trials 4 and 5 and 1.5 for Trial 9.

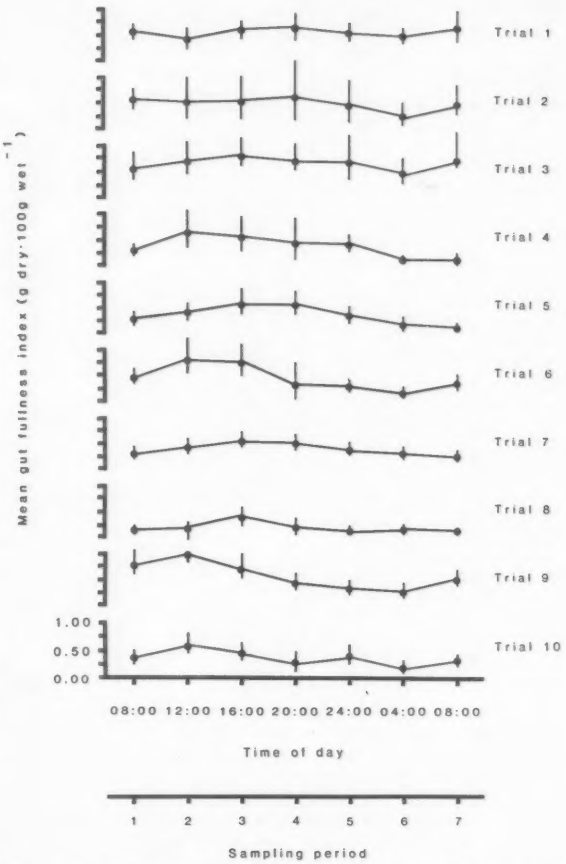

FIG. 2. Daily variability in the content of the complete digestive tract of perch during 10 sampling periods in six lakes. Vertical lines represent 95% CI.

Evacuation Rate (Field)

Field estimates of perch evacuation rates (R) ranged from $0.013 \cdot h^{-1}$ for Trial 1 to $0.117 \cdot h^{-1}$ for Trial 5 (Table 2). R values differed significantly between trials (multiple comparison test for slopes, GT2 method, $p < 0.05$; Sokal and Rohlf 1981). The relationship between field-derived R and water temperature (K) was

(9) $\ln R = 0.150K - 5.79 \qquad r^2 = 0.76.$

Stocking densities expressed both as numbers ($F_{1,6} = 0.30$; $p > 0.50$) and biomass ($F_{1,6} = 0.05$; $p > 0.80$) did not significantly affect evacuation rates determined in the field.

Evacuation Rate (Laboratory)

Laboratory estimates of evacuation rates (R_1) ranged from 0.033 to $0.140 \cdot h^{-1}$ (Table 2) and displayed significant between-

TABLE 2. Evacuation rates and corresponding standard errors obtained from field and laboratory experiments. Corrected field evacuation rate estimates are shown in parentheses.

Field experiments				Laboratory experiments			
Trial	T(°C)	R_i (R'_i)	s_{R_i}	Population	T(°C)	R_1	s_{R_1}
1	10	0.013(0.031)	0.007	3	8	0.035	0.020
2	10	0.018(0.041)	0.009	4	11	0.033	0.009
3	18	0.061(0.095)	0.014	4	15	0.038	0.035
4	23	0.116(0.146)	0.024	3	17	0.067	0.018
5	23	0.117(0.147)	0.016	5	18	0.055	0.018
6	22	0.072(0.098)	0.009	5	20	0.098	0.022
7	18	0.048(0.077)	0.014	3	21	0.140	0.032
8	18	0.016(0.031)	0.010				
9	22	0.114(0.147)	0.014				
10	24	0.095(0.118)	0.110				

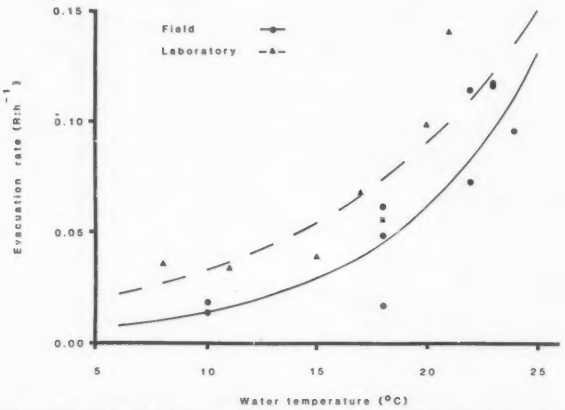

FIG. 3. Relationship between digestive tract evacuation rate and water temperature in field and laboratory evacuation experiments.

experiment differences (multiple comparison test for slopes; GT2 method; $p < 0.05$). The relationship between evacuation rate and water temperature (K) was

$$\ln R_1 = 0.102K - 4.45 \qquad r^2 = 0.73.$$

Stocking densities expressed both as number ($F_{1,3} = 0.05$; $p > 0.80$) and as biomass ($F_{1,3} = 0.02$; $p > 0.80$) did not significantly affect perch evacuation rates.

There was no significant difference between the slopes of equations 9 (field) and 10 (laboratory) describing the relationship between evacuation rates and water temperature (analysis of covariance; $F_{1,12} = 2.86$; $p > 0.10$). The intercepts were, however, significantly different ($F_{1,13} = 9.31$; $p < 0.02$); laboratory-determined evacuation rates were significantly greater than field determined rates at the same water temperature (Fig. 3). We consequently corrected the field estimates of R using laboratory values. This correction increased our field estimates of R by a factor ranging from 1.24 for Trial 10 to 2.38 for Trial 1 (Table 2) and averaged 2.33 for trials performed at 10°C (Trials 1 and 2), 1.70 at 18°C (Trials 3, 7, and 8), and 1.28 between 22 and 24°C (Trials 4, 5, 6, 9, and 10).

The percentage of amphipods in the diet of wild fish ranged from 2 (Trial 3) to 75% (Trials 9 and 10; D. Boisclair and W. C. Leggett, in prep). There was no significant relationship between the correction factor applied to field estimates of R and the percentage of amphipods in the diet of wild fish ($F_{2,3} = 0.76$; $p > 0.05$). We used the corrected R' values in all estimates of perch daily ration.

TABLE 3. Point and simulated daily ration estimates determined with the Eggers and the Elliott and Persson models.

	Point estimates		Simulation estimates	
Trial	Eggers	Elliott and Persson	Eggers	Elliott and Persson
1	0.42	0.52	0.41	0.49
2	0.49	0.42	0.48	0.45
3	1.57	1.85	1.58	1.88
4	1.26	1.27	1.29	1.41
5	1.23	1.21	1.22	1.33
6	1.01	1.01	1.04	1.05
7	0.76	0.77	0.76	0.77
8	0.16	0.16	0.15	0.19
9	1.94	1.75	1.96	1.85
10	0.93	0.94	1.02	0.77

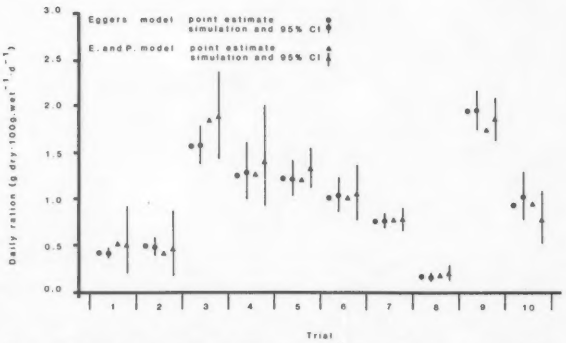
Eggers model point estimate
simulation and 95% CI
E. and P. model point estimate
simulation and 95% CI
Daily ration (g dry 100g wet⁻¹ d⁻¹)
Trial

FIG. 4. Point and simulated daily ration estimates determined with the Eggers and the Elliott and Persson models.

Point and Simulated Daily Ration Estimates

Point estimates of daily ration derived with the Eggers model ranged from 0.16 (Trial 8) to 1.94 g dry wt·100 g wet wt^{-1}·d^{-1} (Trial 9) (Fig. 4). Corresponding values derived with the Elliott and Persson model ranged from 0.16 (Trial 8) to 1.85 (Trial 3).

Mean daily ration as determined by simulation using individual values of gut fullness and evacuation rates ranged from 0.15 (Trial 8) to 1.96 (Trial 9) when using the Eggers model and from 0.19 (Trial 8) to 1.88 (Trial 3) when using the Elliott

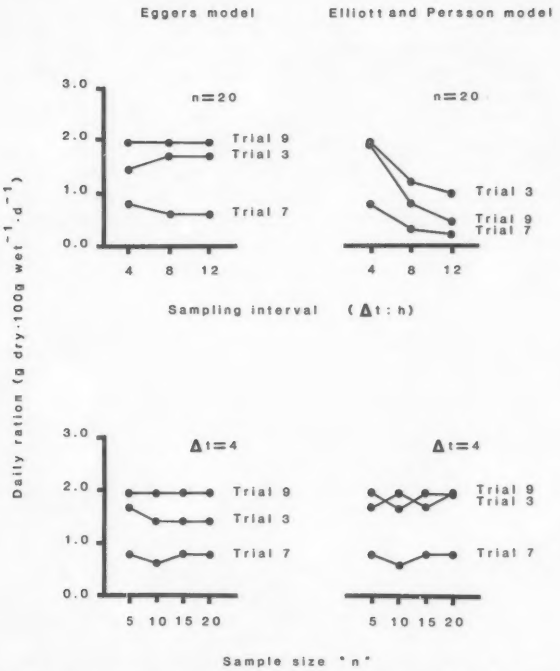

FIG. 5. Effect of different sampling intervals and sample sizes on estimates of daily ration as determined with the Eggers and the Elliott and Persson models.

and Persson model (Fig. 4). Daily ration values derived from simulation did not differ significantly from the point estimates, and the replicated estimates developed for the two models did not differ significantly ($F_{3,1026} = 0.05$; $p > 0.95$).

Effect of Sampling Frequency and Number of Fish Sampled

Only three trials (Trials 3, 7, and 9) had sufficient data (20 fish per sampling period) to allow a test of the effect of number of fish sampled and sampling frequency on daily ration estimates. Daily ration estimated by applying any combination of replicate number and sampling interval to the Eggers model ranged from 1.43 to 1.63 g dry wt·100 g wet wt^{-1}·d^{-1} for Trial 3, 0.64 to 0.81 for Trial 7, and 1.94 to 2.23 for Trial 9 (Fig. 5). A two-way analysis of variance (Arcsin $\sqrt{D}/100$ transformed data) showed that neither the number of fish per sample (Trial 3: $F_{3,688} = 0.53$; $p > 0.60$; Trial 7: $F_{3,688} = 0.93$; $p > 0.40$; Trial 9: $F_{3,688} = 0.11$; $p > 0.70$) nor the length of the sampling interval (Trial 3: $F_{2,688} = 0.11$; $p > 0.80$; Trial 7: $F_{2,688} = 0.93$; $p > 0.30$; Trial 9: $F_{2,688} = 0.26$; $p > 0.70$) had a significant effect on estimates of daily ration obtained with the Eggers model.

Daily ration estimates derived by applying a similar approach to the Elliott and Persson model ranged from 1.00 to 1.95 for Trial 3, 0.25 to 0.81 for Trial 7, and 0.49 to 1.95 for Trial 9 (Fig. 5). Daily ration estimates determined with the Elliott and Persson model were unaffected by the number of fish sampled per sampling interval (Trial 3: $F_{3,129} = 0.33$; $p > 0.80$; Trial 7: $F_{3,124} = 0.86$; $p > 0.40$; Trial 9: $F_{3,130} = 2.31$; $p > 0.05$). These estimates were, however, significantly affected by the time between successive sampling periods (Trial 3: $F_{2,129} = 13.16$; $p < 0.0001$; Trial 7: $F_{2,124} = 43.67$; $p < 0.0001$; Trial 9: $F_{2,130} = 97.17$; $p < 0.0001$). A posteriori comparisons of mean daily ration estimates determined from the Elliott and Persson model showed that values obtained when 4-h sampling intervals were used were consistently and significantly larger (least square means test; $p < 0.05$) than those obtained when 8- or 12-h intervals were employed (Fig. 5).

Discussion

Our analyses indicate that estimates of daily ration developed from the Eggers (1977) model do not differ significantly from those derived by the more general Elliott and Persson (1978) model, even when gut fullness at the beginning and end of a given 24-h sampling period varied by a factor of 2 (Trials 4, 5, and 9). This leads us to conclude that the restriction imposed to date on application of the Eggers model — that the digestive tract contents at the beginning and the end of a 24-h period must be equal — is overly conservative. Eggers (1979) noted that his model (which he humbly, but incorrectly, referred to as Bajkov's 1935 method; see Elliott 1979) is not recommended for piscivorous fish that periodically consume very large prey. Our results do indicate, however, that the Eggers model can be applied to species that feed throughout the day on a wide range of prey types, exhibit occasional feeding peaks, and do not have rigid feeding periodicities.

The major advantage of using the complete digestive tract in the analysis of daily ration is that it gives greater latitude in defining the sampling interval to be used. Elliott and Persson (1978) have shown that the accuracy of daily ration estimated with their model (equation 6 and 7) decreases as the interval between sampling increases. This effect was obvious in our own analysis (Fig. 5) and results from the fact that food consumed during one feeding period may be completely evacuated from the stomach before the next sampling period begins. If this occurs, consumption during this interval is not included in the sequential calculation of $\bar{C}_{\Delta t}$. Use of the complete digestive tract in the analysis reduces this bias because evacuation rates based on the total gut are slower, relative to stomach evacuation rates. Persson (1979), working with perch, reported the relationship between stomach evacuation rate and water temperature to be $\ln R = 0.140K - 4.01$. Our data show the relationship between the evacuation rate of the complete digestive tract and temperature to be $\ln R = 0.102K - 4.45$. Hence, at 20°C the evacuation rate of the complete digestive tract would be 0.09·h^{-1} whereas that of the stomach only would be 0.30·h^{-1}, a threefold difference. At 20°C, evacuation of 50% of a new meal from the stomach would require 2.32 h, compared with 7.70 h for the complete digestive tract. The use of the whole digestive tract thus substantially decreases the probability of missing a meal between two successive sampling periods, and thereby biasing daily ration estimates.

Although part of the difference between our field and laboratory evacuation rates might be due to factors other than stress, a very strong influence of water temperature and food type on evacuation rate (Windell 1978; Persson 1979), which we assessed directly, suggests that stress was the major factor in these differences. Our corrected field estimates of evacuation rates (R'; mean 0.11·h^{-1}) were very similar to independent estimates of R based on a separate field study of perch feeding periodicity (0.10·h^{-1}; Boisclair and Leggett 1985). This similarity gives us confidence that the correction applied was justified and realistic. The advantage of using corrected field rather than laboratory estimates of evacuation rate in our calculation

of daily ration is that it preserves between-lake differences not accounted for by laboratory experiments (i.e. differences in food type, feeding regime, etc.).

The statistical analysis of variation in estimates of daily ration is problematic. This is especially true for comparisons of values derived by different methods. Worobec (1984) and Pennington (1985) developed, respectively, the first formal method for calculating the standard error of estimates derived with the Elliott and Persson (1978) and the Eggers (1977) models.

Three problems restrict the use of the Worobec (1984) formula. First, despite simplifying assumptions about the covariance of successive $\bar{C}_{\Delta t}$ estimates, the calculation of standard errors about Elliott and Persson estimates by this method is complex. Second, as is the case for most techniques used to calculate the standard deviation of the result of an operation (Colquhoun 1971), Worobec's formula is based on the algebraic combination of standard deviations of the operands (s_F and $s_{R'}$). Gut fullness is not a normally distributed variable and must be transformed (Arcsin $\sqrt{F_t/100}$) prior to the calculations. Combination of the standard deviation of an Arcsin $\sqrt{F_t/100}$ transformed variable with that of a nontransformed variable (R') is difficult to interpret. Third, means and standard deviations are not sufficient to test the significance of the difference between daily ration estimates. Also needed are the degrees of freedom, or replicated estimations of daily ration. Given the logistic difficulties of determining even one estimate using the Elliott and Persson model, replication, while possible, is unlikely, especially in the field. These points also apply to Pennington's (1985) formula. In addition, Pennington's formula defines R' as a constant, which is not realistic.

The simulation technique we employed to estimate the standard error around daily ration values avoids the use of complex algebraic formulae and the logistical difficulty of obtaining multiple estimates of R'. Furthermore, this technique is not disabled by lack of normality in the distribution of operands, since the standard deviation of daily ration is determined from the sets of daily ration estimates obtained rather than from the standard deviation of the operands.

The 95% confidence intervals estimated from sets of daily ration values based on the Eggers model were consistently smaller than those estimated with the Elliott and Persson model. This probably occurred because the Eggers model requires only one gut fullness value to calculate daily ration whereas the Elliott and Persson model requires seven such estimates. The Elliott and Persson model thus produces seven times fewer daily ration estimates per trial than does the Eggers model. This represents a clear statistical advantage in favor of the Eggers model.

Our sensitivity analysis indicates that the Eggers model is very robust to changes in sampling schedule. As few as five fish sampled at 12-h intervals yielded daily ration estimates that did not differ significantly from those derived using our original sampling schedule (20 perch per sample at 4-h intervals). The stability of daily ration estimates derived with the Eggers model is probably related to its simplicity. Such robustness was not attained with the Elliott and Persson model. Daily ration estimates decreased as the sampling interval increased. This property, which is related to the mechanical basis of the model, is discussed at length in Elliott and Persson (1978).

Despite the advantages of the Eggers method, we do not recommend that workers interested in estimating daily ration collect only five fish every 12 h over a 24-h period. It would be more prudent to distribute sampling effort on strategic periods of the day depending on the species studied. For benthivorous/planktivorous fish such as perch, a reasonable sampling schedule might involve sampling 10 individuals at approximately 8-h intervals. The Eggers model is not recommended for evaluating the quantity of food consumed by fish during a specific period of a day. For this, the Elliott and Persson model is more appropriate because it is based on sequential estimation of $\bar{C}_{\Delta t}$. The results and tests presented in this paper do indicate, however, that the Eggers model combines logistic simplicity and robustness with precision equal to that obtainable with the more complex Elliott and Persson model. These attributes permit simplified sampling procedures and, consequently, more frequent estimates of daily ration.

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Promotion of Rapid Growth of Rainbow Trout (*Salmo gairdneri*) by a Recombinant Fish Growth Hormone[1]

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The rainbow trout (*Salmo gairdneri*) growth hormone (GH) cDNA clone isolated previously programs the synthesis in *Escherichia coli* of a chimeric GH polypeptide that differs from the mature form of the natural hormone by an additional 11 amino acid residues at the NH_2-terminus. Treatment of yearling rainbow trout with an enriched preparation of this recombinant GH resulted in enhancement of growth. Specific growth rates, weight increase, and length increase of GH-treated fish were markedly higher than those of the mock-treated or untreated fish. In addition, the chemical composition and ultrastructure of muscle tissue of GH-treated fish were not significantly different from that of control fish. These results suggest that the recombinant GH possesses growth-promoting activity and that GH-treatment results in not only accelerated but also normal growth of fish.

Le clone du cADN de l'hormone de croissance de la truite arc-en-ciel (*Salmo gairdneri*) déjà isolé, programme chez *Escherichia coli* la synthèse d'un polypeptide chimérique de l'hormone de croissance. Ce polypeptide, qui diffère de la forme mature de l'hormone naturelle, possède 11 autres résidus d'acide aminé à la terminaison NH_2. Le traitement de tacons O+ de truite arc-en-ciel avec une préparation enrichie de cette hormone de croissance recombinante a entraîné une stimulation de la croissance. Les taux de croissance et les augmentations de poids et de longueur caractéristiques des poissons non traités ou traités à blanc. De plus, la composition chimique et l'ultrastructure des tissus musculaires des poissons traités avec l'hormone de croissance n'était pas significativement différentes de celles observées chez les poissons témoins. Les résultats portent à croire que l'hormone de croissance recombinante peut stimuler la croissance et que le traitement à l'hormone de croissance entraîne non seulement une croissance accélérée mais aussi une croissance normale chez le poisson.

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It was 40 yr ago when Pickford and Thompson (1948) first demonstrated that administration of mammalian growth hormone (GH) to killifish (*Fundulus heteroclitus*) results in a significant increase in body weight and length. Pickford (1954) later showed that growth in killifish could be abolished by hypophysectomy and restored by GH administration. During the last decade, GH has been successfully purified from several fish species (e.g. Farmer et al. 1976, 1981; Cook et al. 1983; Wagner et al. 1985), but unlike its mammalian counterpart, its biological actions in fish have yet to be studied extensively. One reason for the slow progress in this area is the difficulty in obtaining sufficient quantities of purified hormone.

Recombinant DNA technology provides a means to produce biologically active polypeptide hormones in bacteria (Goeddel et al. 1979a, 1979b; Olson et al. 1981; Jay et al. 1984). Recently, we isolated a clone from a cDNA bank prepared from pituitary RNA of rapidly growing rainbow trout (*Salmo gairdneri*) (Agellon and Chen 1986). This clone (pAF51) directs the synthesis of a chimeric GH in *Escherichia coli*. We now report studies conducted to determine the growth-promoting activity of the recombinant GH in rainbow trout. We also provide results to show the potential application of this hormone in the promotion of rapid growth of cultured fish.

Materials and Methods

Fish and Rearing Conditions

Yearling (77.04 ± 13.4 g, 18.47 ± 1.1 cm) and fry (1.32 ± 0.6 g, 4.50 ± 0.9 cm) of rainbow trout were purchased from Spring Valley Trout Farm (Waterloo, Ontario). Fish were kept in fiberglass tanks supplied with continuous flowing dechlorinated water (10°C) and were fed daily with trout feed (Martins 84G: protein, 40%; fat, 10%; fiber, 3%; Martin Feed Mills, Elmira, Ontario). Three weeks prior to the hormone treatment, fish were acclimated to 15°C. A 12L:12D photoperiod was maintained throughout the experiments.

Enrichment of GH Activity in Bacterial Extracts

Escherichia coli HB101 cells containing pAF51 were grown in L-broth containing ampicillin. Cell pellets from overnight cultures were resuspended in buffer containing 50 m*M* Tris–HCl (pH 9.0), 10 m*M* EDTA, and 1 m*M* phenylmethanesulfonyl fluoride and then lysed by treatment with lysozyme (1 $mg \cdot mL^{-1}$) and 0.2% deoxycholate. The viscosity of the cell lysate was reduced by sonication. Cell debris was removed, and the supernatant was adjusted to 1% polyethyleneimine to precipitate nucleic acids. The recovered supernatant was adjusted to 20% saturation with ammonium sulfate. Precipated proteins were collected by centrifugation, dissolved in 6 *M* urea containing 5 m*M* dithiothreitol, and then dialyzed overnight against 30 m*M* ammonium bicarbonate buffer (pH 9.0). The insoluble material present after dialysis was discarded. The soluble fraction (GH-enriched fraction) was used for hormone treatment. The corresponding protein fraction from *Escherichia coli* HB101 containing nonrecombinant plasmid pUC8 was also prepared. Protein concentration of final cell extracts was determined according to standard procedures (Lowry et al. 1951; Bradford 1976) and the amount of recombinant GH was determined immunologically on microtiter plates.

Assay for Growth Promoting Activity

The growth-promoting activity of the recombinant GH was determined by an in vivo assay. Variability in weight and length between groups of test and control fish was assessed by analysis of variance prior to treatment. Protein extracts prepared as described above were dialyzed against 0.9% NaCl before administration to fish.

In the first experiment, groups of 16 yearling fish acclimated to 15°C were given five weekly intraperitoneal injections of GH-containing bacterial extract at a dose of 0.2, 1, and 2 μg GH·g body $weight^{-1}$. These groups are hereafter referred to as the "low-dose," "medium-dose," and "high-dose" groups, respectively. Two additional groups were used as controls. The "mock-treated" group received injections of control bacterial extract at an equivalent concentration of protein administered to the high-dose group; the other group was not treated. All groups were fed to satiation twice daily beginning the day after treatment. In all cases, one tank was used per treatment. Eight weeks after the initial treatment, the experiment was terminated and tissues from fish in each of the experimental and control groups were collected for composition analysis and electron microscopy. Specific growth rates for fish in the first experiment were calculated using the formula $((\ln(WT) - \ln(wt)/T - t)) \times 100$ (see Gill et al. 1985) where WT and wt are initial and final weight, respectively, and T and t are initial and final time (days), respectively. Condition factor, a term relating weight and length, was calculated according to the formula described by Brown (1957) ($CF = 1000W/L^3$ where W is weight (grams) and L is length (centimetres) except that a factor of 1000 instead of 100 was used.

In the second experiment, fry of rainbow trout were given one treatment of GH by the dip method (H. Kawauchi, pers. comm.). Fry ($n = 15$ per group) were prepared for hormone treatment by incubation with a 3.5% NaCl solution for 2 min and then transferred to 0.9% NaCl solution containing 50 or 500 $\mu g \cdot L^{-1}$ of recombinant GH. Since no differences were observed in the growth rates of the two control groups used in the preceding study, control fish in the dip experiment were subjected to osmotic shock in the absence of bacterial extracts. The control group was transferred to a 0.9% NaCl solution. After 30 min, the control and GH-treated fry were rinsed with tank water and then transferred to separate compartments of one holding tank. All treatments were done at 15°C. Fish were fed to satiation twice daily. Weight and length of fish in both groups were scored before and 5 wk after GH treatment.

Chemical Composition Analysis

Muscle tissues dissected from the trunk just anterior to the dorsal fin of selected fish from each of the GH-treated and control groups were analyzed for moisture, total lipids, proteins, and ash according to standard procedures (Bligh and Dyer 1959; Horowitz 1980).

Electron Microscopy

Small portions of liver and myotomal muscle were dissected from GH-treated and control fish. Tissues (1 mm^3) were immersed immediately in 2% glutaraldehyde fixative in 0.1 *M* sodium cacodylate buffer (pH 7.4) containing 0.25 *M* sucrose. After 1 h of fixation at room temperature, the tissues were rinsed then postfixed in 1% buffered osmium tetraoxide for another hour. The tissues were then dehydrated through ethanols, to propylene oxide, and embedded in Spurr's resin. Silver–gold sections, stained with uranyl acetate and lead citrate, were observed at 60 kV in a Philips 300 electron microscope.

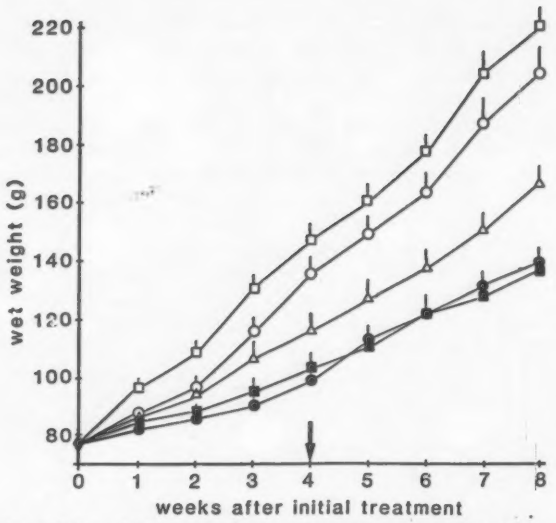

FIG. 1. Effect of GH on growth of yearling rainbow trout. Wet weights of GH-treated and control fish are shown (mean + SE). Open symbols, GH-treated fish: ○, low dose, 0.2 μg·g⁻¹; □, medium dose, 1.0 μg·g⁻¹; Δ, high dose, 2.0 μg·g body weight⁻¹. Closed symbols, control fish: ■, mock-treated fish; ●, untreated fish. The arrow indicates the time of the last treatment.

Results

Enrichment of the Recombinant GH

Immunobinding assays of cell extracts on microtiter plates revealed that the amount of recombinant GH produced by clone pAF51 constituted less than 1% of the total bacterial proteins. Initial fractionation experiments indicated that the majority of recombinant polypeptide was localized in soluble extracts and could be recovered by precipitation with ammonium sulfate at 20% saturation. After dialysis of the GH-containing extract against ammonium bicarbonate (pH 9.0), the majority of GH immunoreactivity remained in solution. Approximately 25% of the total protein in this preparation was determined to be recombinant GH.

Biological Activity of the Recombinant GH

Weight and length of test and control fish were not significantly different prior to hormone treatment ($p > 0.5$). Administration of the recombinant GH by injection led to growth enhancement which was evident as early as 7 d after the initial hormone treatment (Fig. 1). Recombinant GH at a dose of 0.2 $\mu g \cdot g^{-1} \cdot wk^{-1}$ was sufficient to stimulate a significant increase in weight but the highest weight increment was observed in fish which received GH at a dose of 1 $\mu g \cdot g^{-1} \cdot wk^{-1}$. At week 5, 1 wk after the termination of hormone treatment, the low- and medium-dose groups gained 97 and 101%, respectively, over their initial weight whereas the high-dose group gained only 71%. The control groups gained 42% over the same period. Increase in length was less dramatic but statistically significant (data not shown). Over the same period, the GH-treated groups increased in length between 14 and 19%, while both controls increased by only 10.5%.

The specific growth rates of GH-treated fish and control fish were compared during the experiment. During the first 5 wk, the low- and medium-dose groups grew at a rate nearly twice that of the controls, while the high-dose group grew about 1.5 times faster than the controls (Table 1). After termination of hormone treatment (weeks 5–8), the specific growth rates of both the low- and medium-dose groups declined to approximately 1.4 times that of the controls. The growth rate of the high-dose group was approximately 1.3 times that of the controls. The specific growth rates of both control groups were unchanged throughout the experiment. At week 8 the overall weight increase for the low-, medium-, and high-dose groups were 170, 175, and 124%, respectively. The weight gain of the low- and medium-dose groups is not significantly different. The untreated and mock-treated control groups increased 81 and 80%, respectively. Similarly, the overall length increase for the low-, medium-, and high-dose groups were 29, 30, 23%, respectively, and 18% for both control groups. No significant differences in final weight and length were observed between control groups.

TABLE 1. Specific growth rates of GH-treated and control fish during and after GH treatment.

Treatment	Specific growth rate (%·d⁻¹)[a] During treatment (weeks 0–5)	Specific growth rate (%·d⁻¹)[a] After treatment (weeks 5–8)
Uninjected control	1.01	1.00
Mock-treated control	1.01	1.05
GH (0.2 μg·g⁻¹)	1.93	1.44
GH (1.0 μg·g⁻¹)	2.00	1.41
GH (2.0 μg·g⁻¹)	1.53	1.27

[a]Specific growth rates were calculated at weekly intervals and averaged over the first 5 wk of treatment and over the next 3 wk beginning the week after the termination of hormone treatment.

The change in condition factor was another notable observation. One week after the last GH treatment, the condition factors for the low- and high-dose groups increased from 11.80 ± 0.8 to 14.76 ± 0.8 and from 11.90 ± 0.8 to 14.22 ± 1.0, respectively, an opposite effect from what is normally observed in GH-treated salmon (Higgs et al. 1975; Markert et al. 1976; Gill et al. 1985). Thus, GH treatment of rainbow trout, compared with salmon, appears to induce a more significant increase in weight than in length. However, since the natural and recombinant hormones have minor structural differences, the effect on condition factor may be due to a modification of the biological activity.

Differences in behavior and morphology were also noted between experimental and control groups. Fish in the low- and medium-dose groups showed increased appetite and displayed aggressive feeding behavior. Excess food left at the bottom of the tank was usually consumed within 3 h after feeding. In contrast, fish in the control groups ingested less food and did not consume the excess food.

The most significant changes in morphology and behavior were observed in the high-dose group. All fish in this group acquired abnormally dark skin coloration after the first treatment. These fish also were lethargic for up to 24 h after treatment and were less active feeders compared with the two other treated groups. Bulging eyes (exophthalmus) were noted in four individuals and lesions resembling early stages of fin rot were noted in five other fish. These effects subsided after the fourth treatment (week 3). Skin pigmentation returned to normal and was comparable with the controls. These fish also became more active swimmers, particularly during the dark photoperiod, and

TABLE 2. Effect of GH-treatment on growth of rainbow trout fry. Groups of rainbow trout fry ($n = 15$) were subjected to osmotic shock in the presence or absence of GH. Weight was measured prior to and 5 wk posttreatment. Differences between mean weights of GH-treated and control groups were evaluated using Student's t-test. Mean weights were considered to be significantly different if $p < 0.01$. *Significantly different from the GH-treated groups ($p < 0.01$); **no significant difference between these groups; ***no significant difference between these two treatments.

Treatment	Weight (g, $X \pm$ SD) of GH-treated fry		
	Initial	Final	% gain
Saline control	1.33 ± 0.6**	3.94 ± 1.8*	196
GH (50 μg·L^{-1})	1.29 ± 0.7**	5.51 ± 1.6***	327
GH (500 μg·L^{-1})	1.35 ± 0.7**	5.30 ± 1.3***	293

aggressive feeders in comparison with fish in the other GH-treated groups.

Effect of a Single Treatment of Recombinant GH on Trout Fry

The dip method was adopted for fry, since administration of GH by injection is difficult. Five weeks after the GH treatment, a significant difference in weight was observed between control and GH-treated groups ($p < 0.01$). Fish in the low-dose (50 μg·L^{-1}) and high-dose (500 μg·L^{-1}) groups gained 327 and 293% in weight, respectively, whereas the control group gained 196% in weight (Table 2).

Chemical Analysis of Muscle Tissue

No significant difference in chemical composition was observed between muscle tissue obtained from any of the GH-treated and untreated fish, although muscle from the mock-treated fish had slightly lower protein content (Table 3). These observations indicate that the weight increase observed in GH-treated fish was not the result of increased moisture content.

Ultrastructural Studies of Muscle

A major concern of this study is the extent to which GH-treated fish may grow abnormally or exhibit any unusual structure or physiology. Although our sampling was limited to muscle and liver tissues, and was not extensive, the ultrastructural observations were consistent. The liver, a major target organ of GH, exhibited no gross anatomical changes, but did show marked ultrastructural changes in response to injected recombinant GH (Fig. 2A and 2B). Control and experimental livers both have regions of dense glycogen accumulation as well as scattered deposits in other cells. Also, the amounts of both granular and agranular endoplasmic reticulum are similar in control and experimental liver cells. The one dramatic and consistent difference observed is the nature of lipid vesicles seen in control and in GH-treated liver sections. Whereas lipid vesicles are present and reasonably abundant in control liver cells, they are seen in exaggerated amounts and in altered form in the experimental tissues. Most GH-treated liver cells contain profuse clusters of highly electron-lucent vesicles (Fig. 2B). Even in the absence of quantitative studies, this difference is unmistakable, and there is an apparent qualitative correlation between GH dose and the abundance of these vesicles.

Sections of control or GH-treated striated muscle, the other GH target considered in this study, showed no significant differences in histological or ultrastructural organization (Fig. 2C). Significant amounts of glycogen were observed between and within individual myofibrils of GH-treated fish, and some lipid vesicles were observed at the periphery of muscle fibers (Fig. 2C), but these observations are also true of muscle sections taken from control fish.

Discussion

Effect of GH Treatment

The main objective of the present study was to determine whether the recombinant rainbow trout GH was biologically active. Weight gain and length increase of GH-treated and control fish were monitored to determine the growth-promoting activity of the recombinant hormone. In the multiple-treatment study, similar weight gain was observed in the medium- and low-dose groups. However, since the low-dose group received five times less GH than the medium-dose group, the most potent dose used in this study was the low dose. In the single-treatment study, the 50 and 500 μg·L^{-1} doses were equally effective in stimulating growth.

Changes in behavior and morphology were induced by the high-dose treatment. This suggests that unusually high levels of GH in the serum may have adverse effects. Kayes (1977) reported a high mortality rate after treatment of hypophysectomized black bullheads with bovine GH at 10 μg·g body weight^{-1}. Kayes suggested that high doses of bovine GH may cause an allergenic response in fish although he failed to detect antibodies to bovine GH in the serum of sick fish. By contrast, adverse reactions to GH treatment have not been reported for GH-treated salmon. Multiple application of bovine GH at a dosage level of 10 μg·g body weight^{-1} (Markert et al. 1976) or recombinant forms of chicken and bovine GH at 5 μg·g body weight^{-1} (Gill et al. 1985) did not induce any abnormal effects. It is not likely that the adverse effects we observed were caused by toxic components in the bacterial extracts because none of these changes were observed in the

TABLE 3. Percentage of moisture, protein, lipid, and ash in the muscle tissue of GH-treated and control fish. Yearling rainbow trout were treated weekly for 5 wk with control and GH-containing bacterial extract. Four weeks after the withdrawal of hormone treatment, tissue samples were collected from the same region of the carcass of sacrificed fish. Chemical composition analysis was performed after tissue lyophilization. Values shown are corrected to the initial wet weight of the tissue sample.

Treatment	Percent ± SE ($n = 4$)			
	Moisture	Protein	Lipid	Ash
Uninjected control	78.58 ± 1.4	16.54 ± 1.0	3.58 ± 0.5	1.31 ± 0.1
Mock-treated control	80.26 ± 0.8	15.08 ± 0.4	3.48 ± 0.5	1.19 ± 0.1
GH (0.2 μg·g^{-1})	78.91 ± 0.9	15.98 ± 1.0	4.04 ± 0.6	1.17 ± 0.1
GH (1.0 μg·g^{-1})	78.37 ± 0.3	16.58 ± 0.2	3.91 ± 0.5	1.32 ± 0.1
GH (2.0 μg·g^{-1})	79.02 ± 0.9	15.99 ± 0.3	3.89 ± 0.9	1.24 ± 0.1

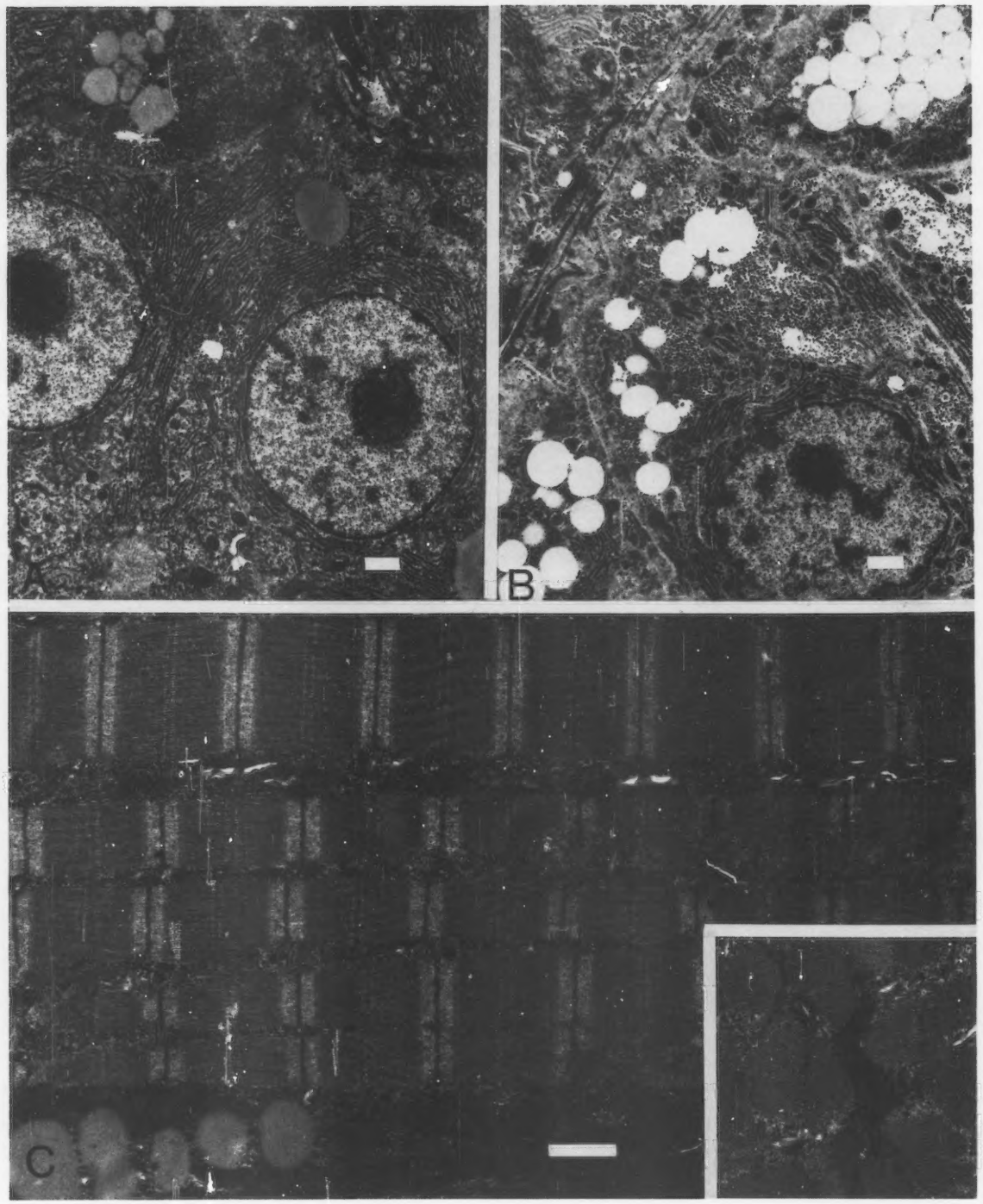

FIG. 2. Ultrastructure of muscle and liver tissue from GH-treated fish. (A) Representative cells from liver of control fish showing abundant endoplasmic reticulum, glycogen deposits, and occasional lipid vesicles; 6500×. (B) Liver cells from fish given medium dose of GH taken 4 wk after treatment; 6500×. (C) Longitudinal section taken through the trunk epaxial muscle just anterior to the dorsal fin; 13000×. Inset shows transversely sectioned muscle fibers. Bars = 1 μm.

mock-treated group which received control protein extract at a mass equivalent to that administered to the high-dose group. However, it is possible that these effects were caused by interaction of the recombinant GH with some unidentified component in the extracts, or by the presence of the additional amino acid residues at the NH_2-terminus of the recombinant GH. These possibilities can be clarified when homogeneous preparations of authentic, chimeric, and unfused recombinant forms of the rainbow trout GH are available.

Stimulated growth in GH-treated fish continued after the termination of hormone treatment, contrary to observations made in mammalian systems. In the case of GH-treated mammals, growth rate appears to decline after termination of GH treatment. In the present study, the apparent maintenance of accelerated growth of GH-treated fish may be a consequence of increased stability of the chimeric GH, or alternatively, due to inefficient clearance of exogenous GH from the serum.

Muscle ultrastructure and composition were similar in GH-treated and untreated fish. However, our results for tissue composition analysis are contrary to those of Higgs et al. (1975). In his study, significant differences in moisture and protein content were found between the muscle of untreated and bovine GH-treated coho salmon. This discrepancy may be due to the time of tissue collection for analysis. Higgs et al. (1975) performed tissue analysis not more than 7 d after the final hormone treatment. In our study, tissue samples were collected 4 wk after the last treatment, and consequently, differences between GH-treated and control fish may have subsided.

Recently, Sekine et al. (1985) demonstrated that unfused chum salmon GH synthesized in bacteria is biologically active and has the same potency as natural chum salmon GH in rainbow trout. The chimeric rainbow trout GH used in our study also promoted accelerated growth in rainbow trout. Thus, the additional 11 amino acid residues at the NH_2-terminal do not appear to abolish the growth-promoting potential of the recombinant rainbow trout GH, although it may alter other biological properties of the hormone. It would be interesting to compare the biological activities of the chimeric and natural GH. However, this may be difficult to perform, since the latter is not readily available.

Future Perspectives

Our results demonstrate that weekly injections of recombinant GH dramatically accelerate the normal growth of yearling rainbow trout. However, GH administration via repeated injections of individual fish is labor intensive and subjects fish to more handling than is ideal. The dip method is a more convenient mode of hormone delivery and is well suited for large-scale operations.

We believe that the recombinant rainbow trout GH can be used in fish farming operations to stimulate rapid growth of salmon and related fishes, but a number of parameters must be addressed before its use may be considered practical. A comprehensive study employing intact and hypophysectomized fish, a pure preparation of recombinant GH, and a more detailed dose regimen must all be undertaken to properly assess the growth-promoting potential of this hormone. Similarly, the effects of both chronic and acute GH treatment, nutrient requirements as well as other rearing conditions affecting GH-treated fish, should be determined in order to maximize growth.

Acknowledgments

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Resident and Dispersal Behavior of Adult Sablefish (*Anaplopoma fimbria*) in the Slope Waters off Canada's West Coast

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Beamish, R. J., and G. A. McFarlane. 1988. Resident and dispersal behavior of adult sablefish (*Anaplopoma fimbria*) in the slope waters off Canada's west coast. Can. J. Fish. Aquat. Sci. 45: 152–164.

A total of 72 735 adult sablefish (*Anaplopoma fimbria*) were tagged and released off the west coast of Canada from 1977 to 1982. As of December 31, 1985, 11 212 (15%) have been recaptured or 16 539 (23%) when recaptures were standardized for differences in recovery effort and unreported recaptures. Off Vancouver Island, relatively few sablefish moved more than 200 km. More movement occurred in Queen Charlotte Sound and off the Queen Charlotte Islands. Thirteen percent of recaptures were made in the United States zone and 96% of these were made in waters off Alaska. Movement was not related to length, age, or sex of adult fish. There was also no relationship between time at liberty and distance travelled; however, there was a trend to move out of the release area with time at liberty. In general, most adults remained within the release area. This resident behavior could result in overfishing because recruitment is dependent on occasional strong year-classes rather than movement from other areas.

De 1977 à 1982, on a étiqueté et relâché 72 735 morues charbonnières (*Anaplopoma fimbria*) adultes au large de la côte ouest du Canada. Le 31 décembre 1985, on en avait recapturé 11 212 (15 %), ou 16 539 (23 %) quand les recaptures sont normalisées afin de tenir compte des différences de l'effort de récupération et des recaptures non signalées. Très peu de morues charbonnières ont parcouru plus de 200 km au large de l'île de Vancouver. De plus grands déplacements ont eu lieu dans le bassin de la Reine-Charlotte et dans les eaux au large des îles de la Reine-Charlotte. Du 13 % des recaptures provenant des eaux américaines, 96 % ont été effectuées dans les eaux au large de l'Alaska. Les déplacements ne sont pas liés à la longueur, l'âge ou le sexe des poissons adultes. De plus, il n'existe aucune relation entre le moment du lâcher et la distance parcourue; toutefois, il existe une tendance de sortie de la zone de lâcher en fonction du moment de lâcher. En général, la plupart des adultes sont demeurés à l'intérieur de la zone de lâcher. Ce comportement sédentaire peut menerà une surpêche car le recrutement dépend d'importantes classes annuelles fortuites au lieu de migrations d'autres régions.

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Sablefish (*Anaplopoma fimbria*) are a long-lived fish (Beamish and Chilton 1982; McFarlane and Beamish 1983b) that occur along the continental slope from California to Alaska. In the Canadian fishery they are captured using baited traps that are set on bottom at average depths of 500–1500 m. The landed value of sablefish increased from less than 1 million Canadian dollars in 1977 to approximately 14 million Canadian dollars in 1985 and currently is the second most important groundfish fishery on Canada's west coast.

In anticipation of increased fishing effort, a tagging study was initiated in 1977 to determine if adult sablefish should be managed as one stock within the Canadian zone and if there was significant movement between Canadian and United States fishing zones. Although tagged fish are still being recovered, the current number of recoveries is relatively small compared with recaptures in previous years. Accordingly, it is appropriate to report the results of the tagging study for adults (55 cm and greater) at this time. Juveniles were tagged later in the study and are still being recovered in large numbers. Thus, the results of the juvenile tagging studies will be reported later. Preliminary information on the movement of juvenile sablefish indicates that they do not behave in a manner similar to adults (Beamish and McFarlane 1983).

Adult sablefish that were recovered within 200 km of the release area were considered resident and those that move beyond 200 km were considered dispersing. Although recoveries have been made for up to 9 yr, it might be argued that the length of the study is not sufficient to indicate long-term movements when the age of sablefish can exceed 50 yr. We show, however, that over a 9-yr period, fishing and natural mortality remove about 90% of all adult fish in a cohort. Thus, relative to the known longevity, our study may not describe long-term movements, but relative to the exploited population, we have studied the movements of most fish throughout their normal life span.

Materials and Methods

Fishing Methods

Most fish were captured using collapsible rectangular traps

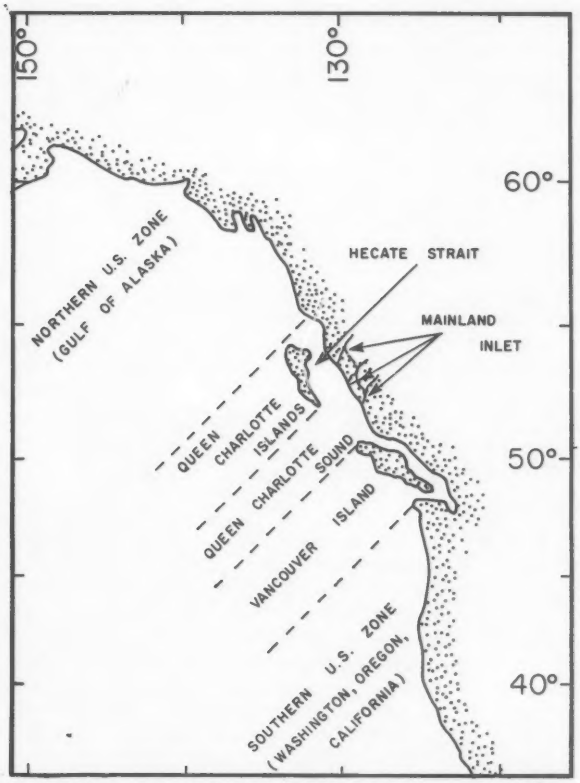

FIG. 1. Major areas used to describe movements of tagged sablefish.

(approximately 85 × 85 × 2.4 m) that were equipped with two tunnels and baited with 5 kg of frozen Pacific herring (*Clupea harengus pallasi*). Korean-style, circular traps with one tunnel were also used. Traps were set on bottom in strings of 15–30 traps at average depths ranging from 300 to 700 m. They were fished for approximately 24 h according to the procedures described by Low et al. (1976) and Webb and Lockner (1973).

Tagging and Recapture

A Floy FD-68 anchor tag was applied according to procedures described in Beamish et al. (1978, 1979). Approximately 10% of the fish received a second "suture" tag (White and Beamish 1972). On the fishing vessel, fish were transferred directly into holding tanks that had a continuous supply of seawater. Most fish were measured for fork length, tagged, and released immediately. Initially, fish were anaesthetized using MS 222 (ethyl aminobenzoate methane sulfonate), but its use was discontinued after several cruises when it was found that fish could be easily handled without anaesthetic.

As part of the recovery procedure, letters describing the tagging experiment and offering a reward were sent to Canadian and foreign fishermen. Fishermen were asked to return the whole fish and were compensated for all reasonable expenses. Recovered tagged sablefish were measured for fork length, examined for sex and maturity, and sampled for otoliths.

Comparisons of Recaptures Among Areas and Years

Tag recaptures were recorded for the five major areas in Fig. 1 in the following categories: less than or equal to 50 km, less than or equal to 200 km, and more than 200 km from the release area. In the three recovery areas in the Canadian zone, we standardized the number of recaptures using estimates of catch per unit of effort (Table 1). Catch per unit effort was used instead of effort because catches will not always be directly proportional to effort. For example, if two areas of different population size receive equal fishing effort, it is probable that catch rates will be higher in the area with more fish. If the rate of movement into these areas from a release area is equal, the higher catch per unit effort in the one area will capture more tagged fish, and if only effort is used to standardize recaptures, it will be assumed erroneously that the rate of movement into this area is greater. All catch per unit effort values were standardized to the 1978 Queen Charlotte Sound values, so that the correction factors in the Canadian zones equalled CPUE in year i divided by CPUE in Queen Charlotte Sound in 1978.

TABLE 1. Catch per unit effort in the Canadian zone (kg/trap).

Year	Vancouver Island	Queen Charlotte Sound	Queen Charlotte Island
1978	25.2	11.6	20.1
1979	14.1	14.0	20.9
1980	15.0	18.5	13.5
1981	10.3	17.9	18.5
1982	11.8	16.6	24.8
1983	13.7	17.0	17.9
1984	11.7	15.0	12.7
1985	15.2	20.3	19.6

In the United States zone, it was not possible to obtain effort or catch per unit effort values for all years. Instead, we used the relationship of average catch per biomass in the United States zone compared with average catch per biomass in the Canadian zone for the combined years 1979 to 1982 as follows:

$$\frac{\text{Catch}_{\text{Can}}}{\text{Biomass}_{\text{Can}}} \times \frac{\text{Biomass}_{\text{US}}}{\text{Catch}_{\text{US}}} = \text{correction factor.}$$

We used the average biomass off the states of Washington and Oregon from 1980 to 1982 (Francis 1985) to represent the average biomass from 1979 to 1982. Dividing the average biomass and catch off the Canadian zone by the average biomass and catch off the United States zone produces a constant correction factor of 0.83.

In the Gulf of Alaska, most recoveries were made in the central and southern areas. The biomass from these areas was obtained from Fujioka (1985) and excluded the estimates in waters shallower than 200 m because the biomass estimates at these depths were considered unreliable (Fujioka 1985). Furthermore, in the Canadian zone, almost no fishing occurs at depths shallower than 200 m (McFarlane and Beamish 1983b), indicating that few adult fish occur here. Dividing the average biomass and catch off the Canadian zone by the average biomass and catch in the Gulf of Alaska produced a correction factor of 1.67.

The number of tag recaptures was also standardized for unreported recaptures. In the Gulf of Alaska we used the nonreporting rates developed by Bracken (1983) and Sasaki (1985). In the central and western portions it appeared from these studies that approximately half of the recaptures were reported. Accordingly, recaptures were standardized by a factor of 2. In the Canadian zone and the southeastern portion of the Gulf of Alaska, reporting of recaptured fish was high (Beamish and

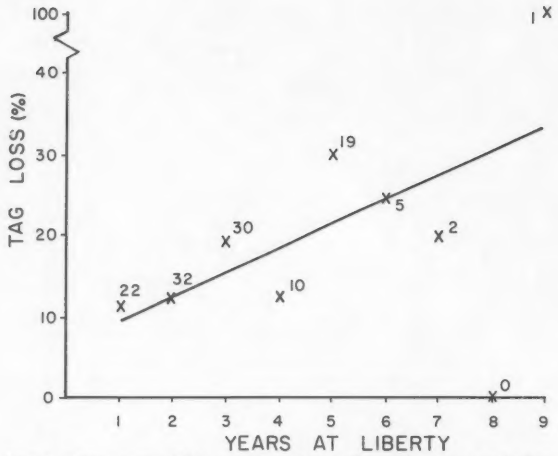

FIG. 2. Percentage of fish recaptured annually with one tag missing. Numbers of fish recaptured with lost tags are indicated for each year at liberty. Line fitted by eye.

McFarlane 1983; Bracken 1983) and required no standardization.

The accuracy of the standardization procedure was assessed by comparing the standardized recapture numbers with the expected number of returns. The expected number of returns was estimated for 2 yr in two of the three release areas using an estimated average annual exploitation rate of 0.1, a natural mortality rate of 0.1 (McFarlane et al. 1985; Funk and Bracken 1983), the rate of tag loss observed in this study, and an estimate of tagging mortality that was guessed to be 0.05. Fish receiving injections of oxytetracycline were not used.

Once the recapture numbers were standardized the percentage movement in and out of the major areas were determined. Each point is a composite number made up of data from a number of release years but the same number of years at liberty. Consequently, the nature of the error structure around each line does not lend itself to statistical hypothesis testing. Therefore, to determine rates of movement, straight lines were fitted by eye.

Results

A total of 72 735 adult sablefish were tagged and released off the west coast of Canada from 1977 to 1982. As of December 31, 1985, 11 212 (15%) of these fish were recaptured (Table 2). Some of these tagged fish were injected with oxytetracycline to mark the otolith as part of a study to validate a method of age determination (Beamish et al. 1983). Injections of 100 and 75 mg/kg produced a high percentage of mortalities (Beamish et al. 1983; McFarlane and Beamish 1987) reducing the overall recovery percentage for the study. If all fish receiving injections of 100 and 75 mg/kg were excluded, a total of 54 916 adult fish were released of which 10 152 (18.5%) were recovered as of December 31, 1985. When all recaptures were standardized (includes oxytetracycline injected fish), a total of 16 539 (23%) were considered recovered.

A total of 5076 fish received two tags. As of December 31, 1985, 822 such fish (16.2%) were recaptured. There was no significant difference in tag loss between the two tag types (*t*-test, $p \geq 0.01$), indicating that the loss of either tag can be considered as a lost tag for an individual fish. This assumes that the rate of loss of either tag is not a result of interaction between the two tags. Using the fitted line in Fig. 2, tag loss in the first year was approximately 10% and after that approximately 2% per year.

Evaluation of Standardization Procedure

One release each year off Vancouver Island in 1979 and in 1980 and one each year off the Queen Charlotte Islands in 1977 and in 1980 were used to compare expected and standardized number of returns. The total number of released fish was 23 060 and the expected number of returns was 6972 (Table 3). The standardized number of returns was 5576 or 20% fewer than expected. If only the release that had been at liberty the longest was used from each area, the expected return was 4510 fish and the standardized number was 4041 or 10% fewer than expected. This close agreement confirms the reliability of the standardization procedure. Consequently, all subsequent analyses use standardized recapture numbers.

Reporting Rates

To assess whether reporting rates changed throughout the study, we compared the annual rate of movement beyond 200 km within the Canadian zone with the annual rate of movement beyond 200 km for the combined Canadian and United States zones. The annual rate of movement greater than 200 km for all years within the Canadian zone was less than the rate for the combined zones (Fig. 3). However, when rates of movement more than 200 km were compared by release years, it was found that for releases from 1977 and 1979 the rates for the Canadian zone and the combined zones (Fig. 4) were similar. Because Canadian fishermen cooperated in this study, we assumed that most fish recaptured in the Canadian zone were reported to us, resulting in a constant reporting rate for the Canadian zone. The assumption of constant reporting in the Canadian zone and similarity of the rates of movement beyond 200 km for 1977 and 1979 releases between the Canadian zone and the combined Canadian and United States zones indicates that the reporting rates within the United States zone for these years did not change.

Standardized Recoveries from Releases off Vancouver Island

From 1977 to 1982, a total of 26 443 fish (including fish injected with oxytetracycline) were tagged and released off Vancouver Island (Table 2). As of December 31, 1985, the recaptures, when standardized for catch per unit effort and corrected for unreported recaptures, were 4258 or 16.1% of the releases (Table 4). Most recaptures (74.5%) were made within 50 km of the release area (Table 4). The annual percentage of recaptures within 50 km of the release area declined at a rate of less than 1% per year (Fig. 5).

A total of 3707 (87%) sablefish were recaptured less than 200 km from the release area (Table 4), and a total of 555 (13%) were recaptured at distances more than 200 km. Fish recaptured at distances more than 200 km within the first year at liberty represented 10% of all recaptures. This percentage increased linearly throughout the study at the rate of 2.2% per year to 28% of all recaptures in the ninth year (Fig. 5).

If the rate of movement beyond 200 km from the release area is constant, then the recapture percentage beyond 200 km for fish at liberty for the same number of years but released in different years should also be constant. To examine this, we compared recaptures from releases from different years (Fig. 6)

TABLE 2. Releases (to 1982) and recaptures (to 1985) of tagged adult sablefish released off the west coast of Canada. A, fish with no injection of oxytetracycline (OTC); B, fish receiving an injection of less than or equal to 50 mg OTC/kg fish; C, fish receiving an injection of 75 or 100 mg OTC/kg fish.

	Releases			Recaptures (%)		
Year	A	B	C	A	B	C
			Vancouver Island			
1977	227		5 278	3 (1.3)		206 (3.9)
1978	1		4 110	0 (0)		375 (9.1)
1979	9 111			1655 (18.2)		
1980	4 703			857 (18.2)		
1982	3 013			501 (16.6)		
Total	17 055		9 389	3016 (17.7)		581 (6.2)
			Queen Charlotte Islands			
1977	5 158			1340 (26.0)		
1978	978		4 427	340 (34.8)		264 (6.0)
1979	6 621			1702 (25.7)		
1980	4 088			399 (9.8)		
1981	3 602	4276	2 552	512 (14.2)	437 (10.2)	90 (3.5)
1982	3 008			474 (15.8)		
Total	23 455	4276	6 979	4767 (20.3)	437 (10.2)	354 (5.1)
			Queen Charlotte Sound			
1978	2		1 452	0 (0)		125 (8.6)
1980	3 110			598 (19.2)		
Total	3 112		1 452	598 (19.2)		125 (8.6)
			Mainland Inlet			
1980	7 019			1334 (19.0)		
Total all areas	50 640	4276	17 819	9715 (19.2)	437 (10.2)	1060 (6.0)

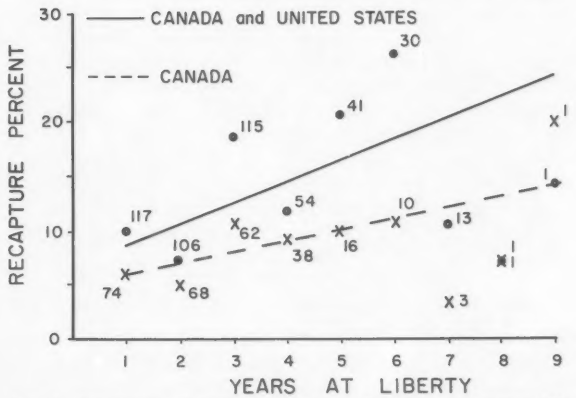

FIG. 3. Percentages of annual recaptures at distances greater than 200 km in the Canadian zone and greater than 200 km in the Canadian and United States zones combined. Numbers of recaptures are indicated for each year at liberty. Lines fitted by eye.

TABLE 3. Expected and standardized number of recaptured sablefish from the earliest releases off the west coast of Queen Charlotte Islands and Vancouver Island.

Year	Number released	Expected number of recaptures as of Dec. 31, 1985	Standardized number of recaptures as of Dec. 31, 1985
		Queen Charlotte Islands	
1977	5 158	1746	2084
1980	4 088	1117	556
		Vancouver Island	
1979	9 111	2764	1957
1980	4 703	1345	979
Total	23 060	6972	5576

but recaptured after the same number of years at liberty. The recapture percentage was constant or increased slightly for fish at liberty for 1, 2, 4 and 5 yr. For fish at liberty for 3 and 6 yr the recapture percentage increased for fish released later in the study. Percentage recaptures for 3 yr at liberty was strongly influenced by the unusually large number of recaptures in 1985. If the 1985 recaptures are excluded, the relationship is similar to the other years. Percentage recaptures of fish at liberty for

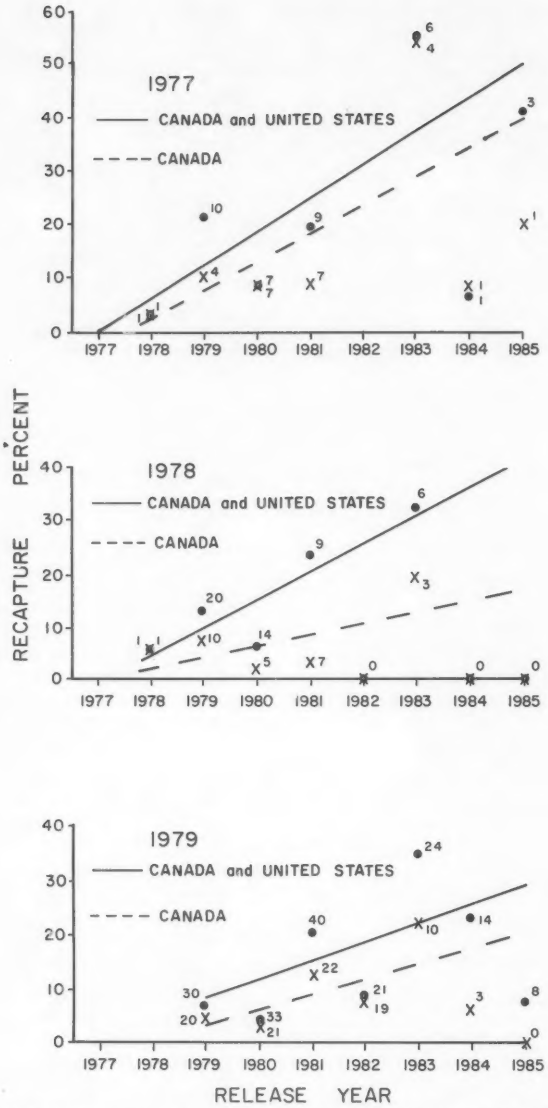

FIG. 4. Percentages of annual recaptures at distances greater than 200 km in the Canadian zone and greater than 200 km in the Canadian and United States zones combined for 1977, 1978, and 1979. Numbers of recaptures are indicated for each recovery year. Lines fitted by eye.

6 yr increased over the release period. This percentage was strongly influenced by the absence of any recaptures of the 1977 releases in 1982.

Standardized Recoveries from Releases in Queen Charlotte Sound and a Mainland Inlet

In 1978 and in 1980, 1454 and 3110 tagged fish, respectively, were released in Queen Charlotte Sound. In 1980, 7019 tagged fish were released in a mainland inlet (Table 2). As of December 31, 1985, the recaptures when standardized were 1094 (24%) from the Queen Charlotte Sound releases and 2015 (28.7%) from the mainland inlet release (Tables 5 and 6).

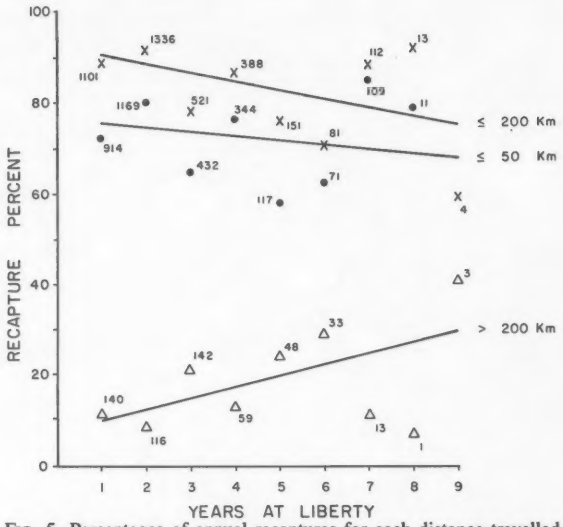

FIG. 5. Percentages of annual recaptures for each distance travelled category for fish released off the west coast of Vancouver Island. Numbers of recaptures are indicated for each distance travelled category and for each year at liberty. Lines fitted by eye.

In Queen Charlotte Sound, most fish (80%) were recaptured within 50 km of the release area. The annual percentage recaptured within 50 km declined from 89 to 69% over the 8-yr recapture period at a rate of 3.1% per year (Fig. 7). A total of 963 (88%) were recaptured less than 200 km from the release area (Table 5), and 131 (12%) were recaptured at distances more than 200 km. The rate at which fish moved out of the release area and farther than 200 km increased at 5% per year from approximately 4 to 40 % (Table 5; Fig. 7).

Most recaptures from the mainland inlet release (77%) were within 50 km of the release area (Table 6). A total of 1654 (82%) were recaptured less than 200 km from the release area and 361 (18%) were recaptured at distances more than 200 km. The annual percentage of fish that were recaptured less than 200 km and less than 50 km from the release area declined sharply (Fig. 8). The rate of decline, however, was strongly influenced by the first year recaptures. If these recaptures are omitted, because the fisherman participating in this study returned the same year to fish this unexploited stock, then the annual percentage that were recaptured less than 200 km and less that 50 km from the release area is almost constant (Fig. 8).

Standardized Recoveries from Releases off the Queen Charlotte Islands

A total of 34 710 fish were tagged and released off the Queen Charlotte Islands from 1977 to 1982 (Table 2). As of December 31, 1985, recaptures, when standardized, were 9098 or 26.6% of the releases (Table 7). The percentage of fish recaptured within 50 km declined from 63 to 32% (Fig. 9) over 9 yr. A total of 7177 (79%) fish were recaptured less than 200 km from the release area (Table 7), and a total of 1921 (21%) were recaptured at distances greater than 200 km from the release area. The annual percentage of fish recaptured beyond 200 km, increased from approximately 20% to 40% at a rate of 2.5% per year (Table 7; Fig. 9).

TABLE 4. Recoveries of tagged sablefish released off the west coast of Vancouver Island by years at liberty and distance travelled.

Years at liberty	No. recaptured (%)							
	Total		>200 km		≤200 km		≤50 km	
	Actual	Standardized	Actual	Standardized	Actual	Standardized	Actual	Standardized
1	973	1241	87 (8.9)	140 (11.3)	886 (91.1)	1101 (88.7)	799 (82.1)	914 (73.7)
2	1211	1452	81 (6.7)	116 (8.0)	1130 (93.3)	1336 (92.0)	998 (82.4)	1169 (80.5)
3	585	663	98 (16.8)	142 (21.4)	487 (83.2)	521 (78.6)	404 (69.1)	432 (65.0)
4	410	447	41 (10)	59 (13.2)	369 (90)	388 (86.8)	325 (79.3)	344 (77.0)
5	175	200	32 (18.3)	48 (24.2)	143 (81.7)	151 (75.8)	116 (66.3)	117 (58.4)
6	103	114	23 (22.3)	33 (29)	80 (77.7)	81(71.0)	70 (68)	71 (62.6)
7	120	120	10 (8.3)	13 (10.6)	110 (91.7)	112 (89.4)	108 (90)	109 (86.1)
8	14	14	1 (7.1)	1 (7.1)	13 (92.9)	13 (92.9)	11 (78.6)	11 (78.6)
9	6	7	2 (40.8)	3 (40.8)	4 (66.6)	4 (59.2)	4 (66.6)	4 (59.2)

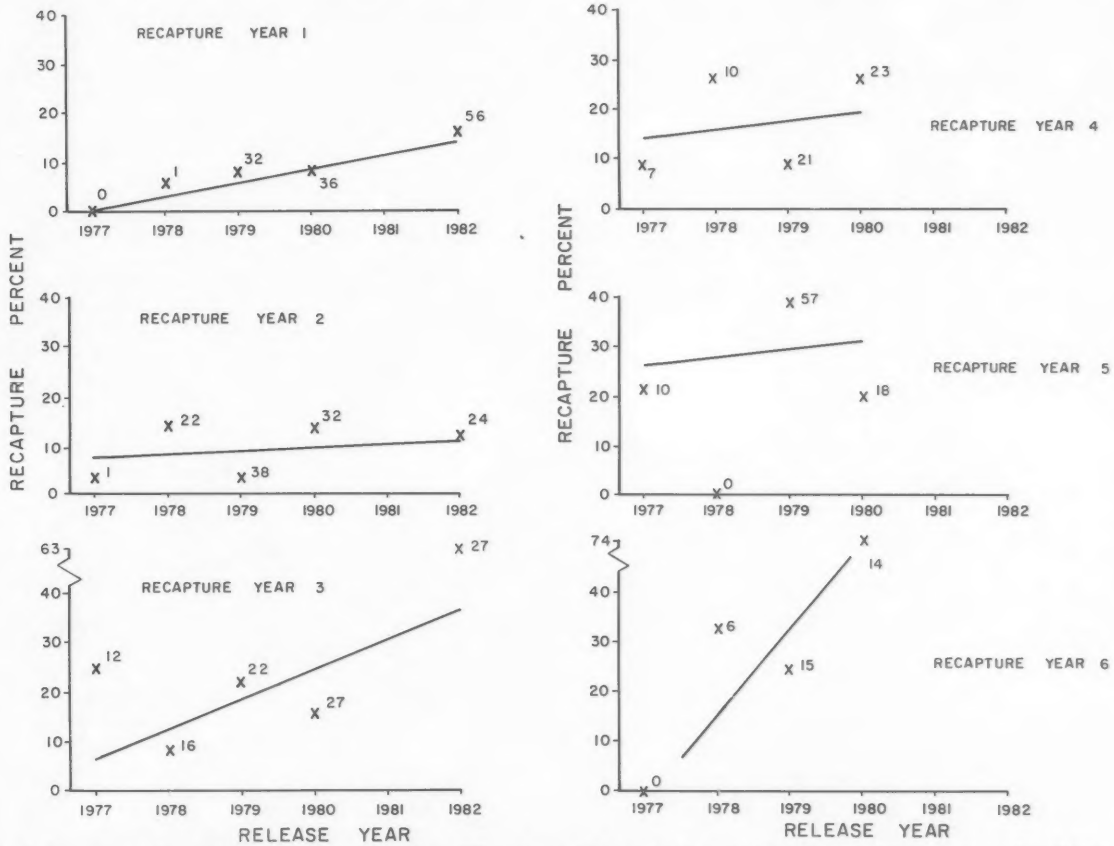

FIG. 6. Percentages of annual recaptures for releases from different years but recaptured greater than 200 km after the same number of years at liberty for fish released off the west coast of Vancouver Island. Numbers of recaptures are indicated for each release year. Lines fitted by eye.

Movements were compared for recaptured sablefish which moved more than 200 km from releases from different years (Fig. 10), but recaptured after the same number of years at liberty, as was done for the Vancouver Island releases. Off the Queen Charlotte Islands the percentage recaptures increased for all years. In most cases a larger percentage of fish that moved beyond 200 km were recaptured from releases made in 1980, 1981, and 1982. This was particularly evident for fish recaptured after 1 and 5 yr where a dramatic increase in recapture percentage occurred from 1980 releases.

Movements of recaptured sablefish from one release off the west coast of Queen Charlotte Islands in 1977 were compared with the exact location of capture and release. A total of 5158 sablefish were released and 2084 (40%) recaptured (Table 3). The average recapture rate within 20 km of the release area decreased from 74 to 25% at a rate of 6% per year. After 9 yr, 45% of these recaptures were still being recaptured within 50 km of the immediate release area.

TABLE 5. Recoveries of tagged sablefish released off Queen Charlotte Sound by years at liberty and distance travelled.

Years at liberty	No. recaptured (%)							
	Total		>200 km		≤200 km		≤50 km	
	Actual	Standardized	Actual	Standardized	Actual	Standardized	Actual	Standardized
1	165	258	7(4.2)	17(6.6)	158(95.8)	241(93.4)	150(91)	229(88.8)
2	265	395	21(7.9)	39(9.8)	244(92.1)	356(90.2)	215(81)	326(82.5)
3	145	228	13(10)	32(14.0)	132(90)	196(86.0)	117(81)	169(74.1)
4	82	123	6(7.3)	15(12.2)	76(92.7)	108(87.8)	65(79)	97(78.9)
5	41	54	7(17)	10(18.5)	34(83)	44(81.5)	29(71)	42(77.8)
6	14	23	7(50)	14(60.8)	7(50)	9(40.2)	7(50)	9(39.1)
7	8	10	3(38)	4(40)	5(62)	6(60)	4(50)	5(50)
8	3	3	0(0)	0(0)	3(100)	3(100)	3(100)	3(100)

TABLE 6. Recoveries of tagged sablefish released in a mainland inlet by years at liberty and distance travelled.

Years at liberty	No. recaptured (%)							
	Total		>200 km		≤200 km		≤50 km	
	Actual	Standardized	Actual	Standardized	Actual	Standardized	Actual	Standardized
1	472	745	2(0.4)	2(0.3)	470(99.6)	743(99.7)	465(98.5)	734(98.5)
2	271	424	65(24)	109(25.7)	206(76)	315(74.3)	187(69)	286(67.5)
3	236	366	59(25)	113(33.6)	177(75)	253(66.4)	157(66.5)	223(60.9)
4	143	217	35(24.5)	61(28.1)	108(75.5)	156(71.9)	94(65.7)	136(62.7)
5	169	221	41(24.3)	58(26.2)	128(75.7)	163(73.8)	120(71)	154(69.7)
6	30	42	11(36.7)	18(42.9)	19(63.3)	24(57.1)	14(46.7)	18(42.9)

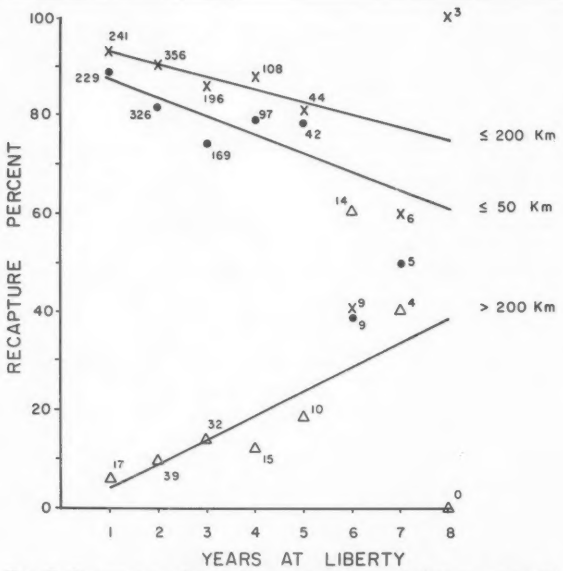

FIG. 7. Percentages of annual recaptures for each distance travelled category for fish released in Queen Charlotte Sound. Numbers of recaptures are indicated for each distance travelled category and for each year at liberty. Lines fitted by eye.

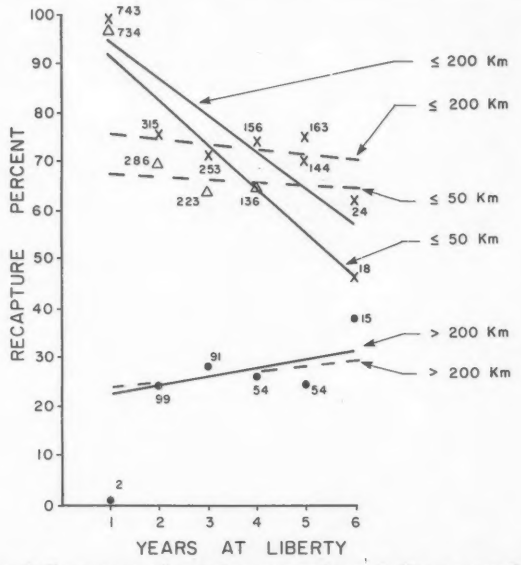

FIG. 8. Percentages of annual recaptures for each distance travelled category for fish released in a mainland inlet. Numbers of recaptures are indicated for each distance travelled category and for each year at liberty. Broken lines identify the relationship if the first year recaptures are excluded. Lines fitted by eye.

Distance Travelled in Relation to Sex, Size, Age, and Time at Liberty

Sex of tagged and released fish could not be determined without killing the fish. Therefore, comparisons of movements of males and females were made by examining sex ratios of recaptured fish. The female to male ratio for fish recaptured within 50 km of the release area was 1.8:1 and for fish recaptured more than 200 km was 2:1.

The movements of males and females were examined separately because mature males grow much less than mature females (Beamish and Chilton 1982; McFarlane and Beamish 1983b). A total of 7992 sablefish that were not tagged were examined for sex during tagging cruises from 1977 to 1980. There were 4790 females of which 40% were less than 70 cm and 60% were greater than or equal to 70 cm. If these per-

TABLE 7. Recoveries of tagged sablefish released off the west coast of the Queen Charlotte Islands by years at liberty and distance travelled.

Years at liberty	No. recaptured (%)							
	Total		>200 km		≤200 km		≤50 km	
	Actual	Standardized	Actual	Standardized	Actual	Standardized	Actual	Standardized
1	1475	2556	150(10)	338(13.2)	1325(90)	2218(86.8)	1120(76)	1900(74.1)
2	2065	3426	264(13)	544(15.8)	1801(87)	2882(84.2)	1415(69)	1534(44.5)
3	1009	1557	219(22)	479(30.8)	790(78)	1078(69.2)	583(58)	915(58.8)
4	463	711	116(25)	240(33.7)	347(75)	471(66.3)	234(51)	334(47.0)
5	268	406	73(27)	150(37.0)	195(73)	256(63.0)	129(48)	190(46.8)
6	155	253	40(26)	92(36.4)	115(74)	161(63.6)	89(57)	133(52.6)
7	73	117	18(25)	38(32.5)	55(75)	79(67.5)	43(59)	68(58.1)
8	30	40	11(37)	19(47.5)	19(63)	21(52.5)	13(43)	16(41.3)
9	20	32	9(45)	21(65.6)	11(55)	11(34.4)	8(40)	9(28.1)

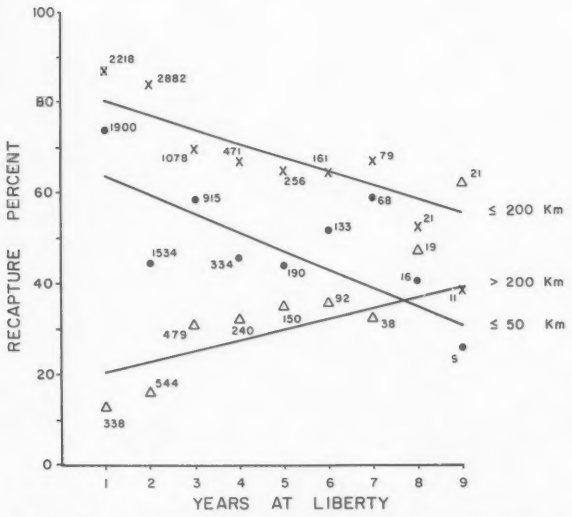

FIG. 9. Percentages of annual recaptures for each distance travelled category for fish released off the west coast of the Queen Charlotte Islands. Number of recaptures are indicated for each distance travelled category and for each year at liberty. Lines fitted by eye.

centages were representative for all areas and all years and if larger females moved farther than smaller females, the percentage larger than 70 cm and recaptured more than 200 km would increase. Because all fish will grow, there will be a tendency to recapture larger fish; however, adult female sablefish grow less than 1 cm/yr, and by restricting the recapture period to 3 yr, we minimize the effect of growth on the analysis. For females that moved more than 200 km, 42% were less than 70 cm and 58% were greater than or equal to 70 cm.

Because adult males grow very slowly and few grow to sizes larger than 65 cm, it is difficult to examine movement in relation to size. There was no difference in the mean size of males that moved greater than 200 km (61.3 cm) and those recovered within 50 km (61.1 cm) of the release area.

There was no significant difference (t-test, $p > 0.05$) in the mean age of male or female fish that moved more than 200 km from the release area and fish recaptured within 200 km of the release area in the Canadian zone. The mean age of male sablefish recaptured within 200 km of the release area was 16 yr, compared with 14 yr for fish recaptured more than 200 km from the release area. Females recaptured within 200 km of the release area had a mean age of 17 yr, compared with 18 yr for those recaptured more than 200 km from the release area.

No relationship was found between distance travelled and time at liberty for all recoveries (Fig. 11).

Movement into United States Zone

A total of 2156 (13.0%) of all standardized recoveries were made in United States waters. Of these, 2070 (96%) were recovered in the Gulf of Alaska. Releases off Vancouver Island accounted for 254 of the recaptures in United States waters, of which 216 (85%) were recovered in the Gulf of Alaska. This, however, represents only 6% of the total recaptures of the total Vancouver Island releases. In contrast, 15.8% of total recaptures from releases off the Queen Charlotte Islands were made in the United States zone. For fish recaptured in United States waters from releases from Queen Charlotte Sound (451) and Queen Charlotte Island (1451), 96 and 98%, respectively, of recaptures were made in the Gulf of Alaska.

Throughout the study the annual percentge of fish that were recaptured in the Gulf of Alaska increased from 9 to 18%. In contrast, the percentage movement from the Canadian zone to waters off the southern United States increased from 0.2 to 2%.

Discussion

Sablefish tagging studies before 1966 (Alaska Department of Fish and Game 1953, 1954; Phillips et al. 1954; Edson 1954; Holmberg and Jones 1954; Pruter 1959; Pasquale 1964) were of limited value because there was no commercial fishery throughout the range of the species. The limited ability to recapture sablefish precluded estimates being made of rates and direction of movement out of the release area.

Although tagging continued after 1966 (Phillips 1969; Pattie 1970; Sasaki 1979, 1980, 1985; Beamish et al. 1980; Beamish and McFarlane 1983; Bracken 1982, 1983; Wespestad et al. 1978), recapture rates remained low except in studies by Beamish et al. (1980) and Beamish and McFarlane (1983). Beamish and McFarlane (1983) and Bracken (1983) were the only ones to standardize recaptures for variation in catch and effort among areas; these authors and Sasaki (1985) also considered unreported recaptures.

In our study, the recapture rate as of December 31, 1985, was 23% including fish receiving oxytetracycline injections. This is considerably higher than previously reported recaptures which ranged from less than 1 to 6.4% (Holmberg and Jones 1954; Novikov 1968; Sasaki 1979, 1980; Wespestad 1983;

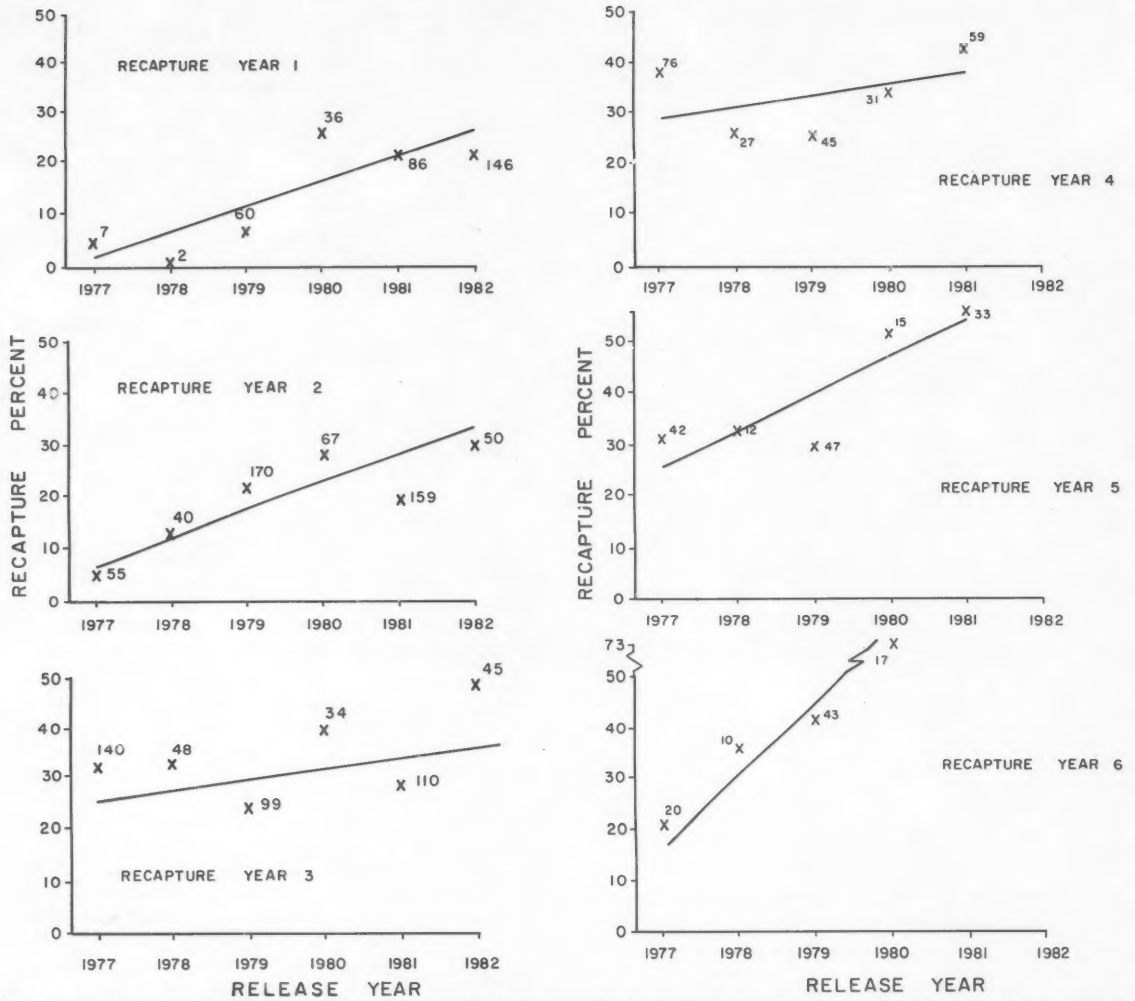

FIG. 10. Percentages of annual recaptures for releases from different years but recaptured greater than 200 km after the same number of years at liberty for fish released off the west coast of the Queen Charlotte Islands. Numbers of recaptures are indicated for each release year. Lines fitted by eye.

Shaw 1986). Our high recapture percentage provided sufficiently large numbers for studying patterns of movement.

All recovery percentages must be directly comparable before rates and direction of movement are determined and compared. For example, an increase or decrease in reporting rate could give the appearance of increased or decreased movement. Because reporting within the Canadian zone was known to be high and probably constant, the rate of movement beyond 200 km within the Canadian zone provides a basis for assessing changes in reporting rate within the United States zone. The annual rate of movement of fish more than 200 km within the Canadian zone was less than movement more than 200 km for the Canadian and United States zones combined. This indicates that either there were gradual increases in number of fish that moved into the United States zone or that there was an increase in the reporting rate in the United States zone. Because the annual rate of movement of fish beyond 200 km was similar for the Canadian zone and the combined Canadian and United States zones for 1977 and 1979 releases, it is unlikely that a change in reporting occurred. Thus, there must have been increased movement into the United States zone. The difference in behavior that occurred for the 1978 releases cannot be explained but cannot be a result of reporting changes because fishermen would not be able to selectively report recaptures of only the 1978 releases.

The accuracy of our standardization procedures was assessed by comparing standardized number of recaptures with the expected number of recaptures. The standardized estimate was 20% less than expected or 10% less than expected for releases that were out the longest. The differences between the standardized and expected numbers are quite small considering that the expected number of recaptures was based on exploitation and natural mortality rates that were assumed to be constant among years and areas and the error associated with these rates was unknown. This close agreement indicates that the standardization procedures were reliable and that the movements

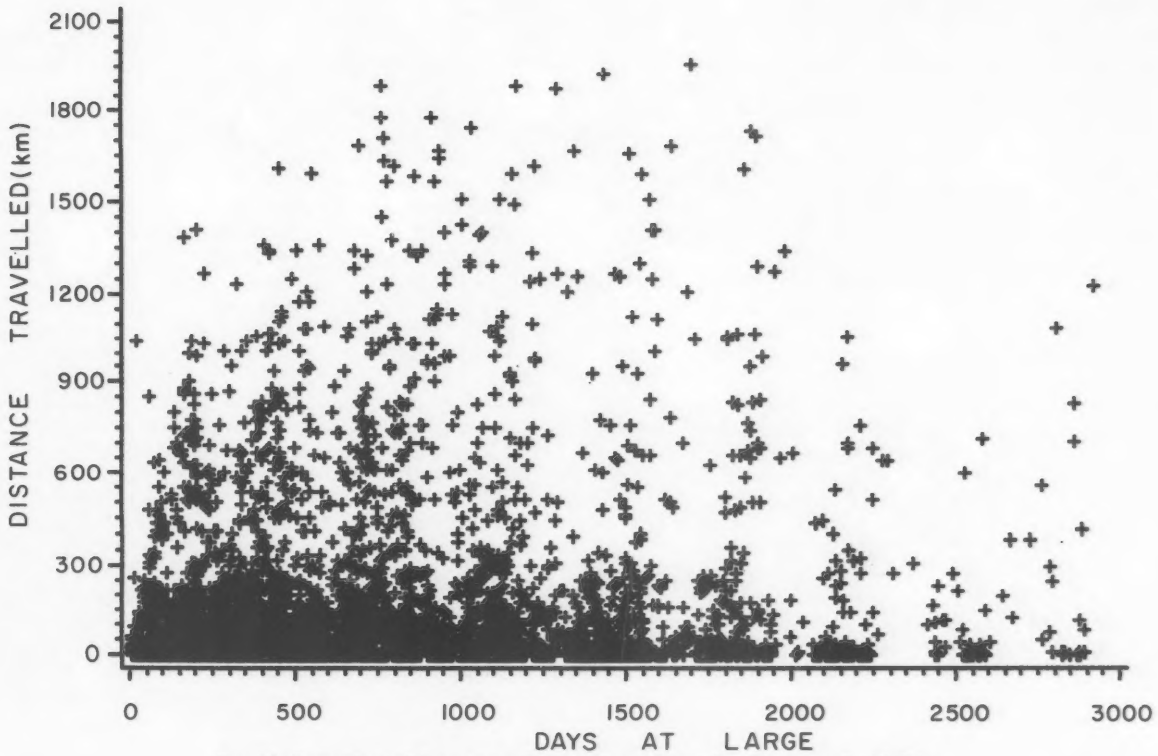

FIG. 11. Comparison of distance travelled and time at liberty for all recaptured sablefish.

described in this study were real and not the result of differences in fishing effort or in the reporting of tag recaptures.

Tag loss and total mortality reduced the number of marked fish in the population. After approximately 9 yr the number of recaptures was small and contributed more to the variance associated with the trends in movement than an improved understanding of these trends (Fig. 5, 9). Accordingly, fish were at liberty for a sufficient time to identify resident and dispersal behavior within the population.

Movements from Release Areas

Off Vancouver Island, there was a slight decline in the annual percentage of fish recovered within 50 km of the release area over the 9-yr period. The percentage of fish that were recaptured more than 200 km from the release area increased at an annual rate of approximately 2%, indicating that there was a small and relatively constant percentage of fish that moved beyond 200 km annually. This behavior was confirmed by examining fish from different release years that were recaptured after the same number of years at liberty. The percentages of fish recaptured at distances more than 200 km after 1, 2, 4, and 5 yr at liberty were almost constant and increased with time at liberty for each of these years. Although there were differences in behavior for fish recaptured after 3 and 6 yr at liberty, the trends for 1, 2, 4, and 5 yr at liberty were quite strong and believed to indicate the true behavior of the population. In general, off Vancouver Island, the percentage of sablefish that moved more than 200 km was small and increased slightly throughout the study.

In contrast, off the Queen Charlotte Islands there was more movement and the rate of movement out of the release area was not always constant. The average annual percentage of fish that were recaptured within 50 km of the release area declined approximately 35% over the study. This compares with a 9% decline observed off Vancouver Island. At the same time, annual percent recaptures of fish beyond 200 km from the release area increased from approximately 20 to approximately 40%. Comparisons of fish from different release years but recaptured after the same number of years at liberty also showed increasing rates of recapture percentage. The difference in behavior cannot be explained but could be partly related to sablefish density. The area of the continental slope (100–1000 m) off the Queen Charlotte Islands is approximately one-half of the area off Vancouver Island, and it is possible that the increasing abundance of sablefish in the Canadian zone (McFarlane et al. 1985) affected the behavior of resident sablefish off the Queen Charlotte Islands more than off Vancouver Island. The limited area of the continental slope off the Queen Charlotte Islands and the recruitment of the large 1977 year-class (McFarlane and Beamish 1983a; McFarlane et al. 1985) may have contributed to the increasing rate of movement.

It was possible to study the behavior of sablefish off the Queen Charlotte Islands in detail in one particular area. Over the period 1977–85 the percentage of fish recovered within 20 km of the exact release area declined in the same manner as observed for all other recaptures, corroborating the observation that a greater rate of movement occurred.

Because there were fewer releases in Queen Charlotte Sound than in other areas, the interpretation of movements was more

difficult. In general it appeared that movements were similar to movements off the Queen Charlotte Islands.

Only one release was made in a mainland inlet. Recapture of these fish at distances greater than 200 km indicated a slight increase in recovery percentages with time, which was similar to the behavior in other areas. As mentioned, the release in this inlet was heavily exploited in the first year when approximately one half of the total recaptures were made. Unfortunately, it was not possible to standardize the recaptures between this localized intensive fishery and catches beyond 200 km. However, if the first-year recaptures were ignored, the annual rate of recapture of fish within 50 km declined slightly, similar to the recaptures within 50 km off Vancouver Island. These observations indicate that most sablefish remained in the inlet.

The inability to demonstrate a relationship between time at liberty and distance travelled was consistent with most other studies (Bracken 1983; Dark 1983). Only Wespestad (1983) reported a slight but significant increase in distance travelled with days at large. We conclude that sablefish will move out of an area with time but there was no relationship between time and distance travelled.

Movement into Waters off the United States

The tendency for adult sablefish to move north was reported earlier (Beamish and McFarlane 1983; Dark 1983). Because recapture data were not standardized in other studies, we cannot compare our estimates of rates of movement in and out of the Canadian zone. It is known, however, that movement of adults into the Canadian zone occurs from the north and the south. For example, in our study we recaptured 48 fish that were released in United States waters to the south of Canada from 1977 to 1983. Shaw (1986) indicated that these amounted to 4% of the total recaptures of fish tagged and released in the United States zone. There also is some indication that adult sablefish at the northern end of their distribution move south. Bracken (1982) reported the recovery of 164 adult sablefish (>60 cm) from releases in the Gulf of Alaska. He reported that 43% moved south and 7% moved north; however, relatively few fish were recovered and his data were not standardized by area. In the area immediately north of the Canadian zone (between 137°W and 54°30′N), Bracken found that most fish (86%) did not move and of those that did, an equal number moved north and south. In contrast, in the northwest Gulf of Alaska (between 147 and 170°W), most adult fish (53 fish; 72%) moved out of the release area, with 51 (96%) moving south and 26 (51%) moving into the Canadian zone (Bracken 1982). Therefore, Bracken's study shows that sablefish at their extreme northern distribution moved into the Canadian zone although those immediately north of the Canadian zone moved very little. It was not possible to determine if sablefish movement resulted in net losses or net gains to the Canadian zone. However, 13% of all recaptures of Canadian releases were made in the United States zone and 96% of these were recaptured in the Gulf of Alaska. Because the biomass of adult sablefish is thought to be four to five times greater in the Gulf of Alaska than in the Canadian zone (Fujioka 1985; McFarlane et al. 1985), a small percentage of movement into the Canadian zone could compensate for the disproportionate northward movement out of the zone.

Movement by Sex, Size, and Age

The sex ratios of recaptured fish recovered within 50 km and those recaptured more than 200 km were almost identical indicating that there was no trend for either sex to move out of the release area at a greater rate. In an earlier study (Mason et al. 1983), we showed that there was no spawning migration; therefore, there does not appear to be short-term or long-term differences in the movement of either sex.

In the Gulf of Alaska, the direction of movement was related to fish length (Bracken 1982). Smaller fish moved north and west; larger fish moved southeast. Although the recapture percentages were not standardized and the sample size was small (164 fish), the data appear convincing. In our releases of tagged juveniles in the Canadian zone, we reported a strong tendency for juveniles to move north (Beamish and McFarlane 1983). More recent observations have confirmed this behavior (McFarlane and Beamish, unpubl. data). For adult fish, we did not observe any relationship between movement and length. The absence of a relationship between fish length and direction of movement was also reported by Shaw (1986) and Wespestad (1983). Dark (1983) demonstrated a relationship between distance travelled and size in some areas; however, he used only two size categories which would not separate adult and juvenile fish. If the observations of Bracken (1982) are real, then the southward movement probably is a result of adults at the northern limits of their distribution moving south.

Movement beyond 200 km in relation to age was difficult to estimate because otoliths (Beamish and Chilton 1982) were difficult to obtain from fish recaptured by foreign fishermen. Thus, most samples came from the Canadian zone. A comparison of the age distribution of adult fish that moved more than 200 km in the Canadian zone with fish recaptured within 200 km of the release area indicated no tendency for older or younger adult fish to move out of the release area at a greater rate.

Resident and Dispersal Behavior

The observation that rate of movement out of some areas increased at a constant rate suggested that there could be a general pattern to the resident and dispersal behavior of sablefish. Our studies and others (Bracken 1982, 1983; Dark 1983; Wespestad 1983; Shaw 1986) all demonstrated that a majority of sablefish are resident. Some fish, however, moved considerable distances, which would ensure that genetic exchange occurs throughout the range and that sablefish are one population. Tsuyuki et al. (1965) were unable to detect genetic differences that would indicate distinct populations. Gharrett et al. (1983), in a preliminary analysis, felt that some stocks could be separated genetically; however, a more extensive comparison (G. Winans and G. Stauffer, NW and Alaska Fisheries Center, Seattle, WA, pers. comm.) has recently concluded that, in general, sablefish stocks are one population.

Our study indicates that throughout their range, the movements of adults will contribute little recruitment to established fisheries. The annual rate of movement varies among areas; however, the biomass of fish that move, relative to the biomass that is fished, is small. The main source of recruitment to any particular area is from juveniles. In particular, occasional strong year-classes are the major source of recruitment. For example, large numbers of sablefish occurred in the eastern Bering Sea in the early 1960s and in the early 1980s (Typanogov and Kodolov 1983). We believe that these sablefish were from strong year-classes produced in the late 1950s and in 1977 (McFarlane and Beamish 1983b). During such periods of strong year-classes, the population expands and areas such as the eastern Bering Sea or inshore waters such as Puget Sound and the Strait of Georgia would be colonized. If conditions are suitable for survival and growth, larval sablefish in these newly colonized

areas will establish new stocks. If conditions for the survival of larvae are not suitable for these stocks and in the absence of successive recruitment from strong year-classes, such stocks are usually overfished.

The tendency for juveniles to move northward has been clearly demonstrated (Beamish and McFarlane 1983; unpubl. data; Dark 1983; Bracken 1982). Bracken (1982) proposed that the northward movement of juveniles was compensated by a southward movement of adults. We conclude that a southward movement of adults could compensate for a northward movement of adults, but not for the movement of juveniles out of the Canadian zone. If there was a compensating movement of adults back into and through the Canadian zone, we should have observed a greater amount of southward movement within the Canadian zone. Because large numbers of juveniles move north, there must be an accumulation of these younger sablefish in northern areas. Because the largest biomass of sablefish appears to be in the southern and central Gulf of Alaska (54°30′N by 154°W), we propose that many of the juveniles that migrate north from the Canadian zone become resident in the southern and central Gulf of Alaska. It also follows that in some location, movements north and south would be equal and that fish in this area would have the lowest dispersal rate. We propose that this area is the southern Gulf of Alaska.

We believe that sablefish constitute one population throughout the range, and within this range most adults are resident. Recruitment is maintained primarily from juveniles and adult dispersal is not a major contributor to the biomass of established fisheries, but does serve to maintain genetic exchange. Recruitment throughout the population and particularly at the extremes of the range is heavily dependent on periodic strong year-classes. Because recruitment is irregular and most adults are resident, localized overfishing can occur even if exploitation rates are relatively low.

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Dynamics of Lake Michigan Plankton: A Model Evaluation of Nutrient Loading, Competition, and Predation[1]

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Lake Michigan's offshore ecosystem has been altered dramatically during the past decade. Summer zooplankton dominance has changed from calanoid copepods to *Daphnia* and the substantial contribution of filamentous blue-green algae to summer phytoplankton has been replaced by phytoflagellates. These changes occurred concurrently with reduced P load, P concentration, and abundance of the dominant zooplanktivore, the alewife (*Alosa pseudoharengus*). In this analysis we pose alternative hypotheses of nutrient loading and species interactions as determinants of zooplankton and phytoplankton species composition in the summer epilimnion. We evaluate these hypotheses with a food web model that was calibrated to measurements of the 1980s Lake Michigan plankton composition and algal production, sedimentation, and growth rates and literature estimates of zooplankton secondary production and nutrient excretion. The model simulates the influence of gradients of both P load and alewife abundance on predation–competition interactions. We conclude that summer plankton composition in Lake Michigan is controlled largely by predation. The model further predicts a return to a plankton community similar to that of the 1970s under a scenario of increasing invertebrate predation by a new zooplankton species for Lake Michigan, *Bythotrephes cederstroemi*.

Au lac Michigan, l'écosystème situé au large a changé de façon spectaculaire au cours des dix dernières années. Les copépodes calanoïdes, qui dominaient auparavant le zooplancton en été, ont fait place aux *Daphnia*, tandis que les cyanophycées filamenteuses, qui contribuaient de façon importante au phytoplancton en été, ont été remplacées par les phytoflagellés. Ces changements se sont produits en même temps que la réduction de la charge en P, de la concentration de P et de l'abondance du gaspareau (*Alosa pseudoharengus*), le zooplanctivore dominant. Dans cette analyse, nous proposons de nouvelles hypothèses concernant l'effet de la charge en éléments nutritifs et des interactions entre les espèces sur la composition spécifique du zooplancton et du phytoplancton dans l'épilimnion en été. Ces hypothèses sont évaluées à l'aide d'un modèle de réseau trophique calibré, d'une part, avec les mesures faites au lac Michigan en 1980 sur la composition du plancton ainsi que sur la production, la sédimentation et les taux de croissance des algues, et, d'autre part, avec les estimations publiées de la production secondaire et de l'excrétion d'éléments nutritifs chez le zooplancton. Le modèle simule l'effet de gradients de la charge de P et de l'abondance du gasparau sur les interactions prédation–compétition. Il ressort que la composition estivale du plancton dans le lac Michigan est en grande partie déterminée par la prédation. De plus, le modèle prédit un retour à une communauté planctonique semblable à celle des années 1970 suivant une prédation accrue des invertébrés par une espèce de zooplancton, *Bythotrephes cederstroemi*, nouvelle pour le lac Michigan.

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Recent changes in Lake Michigan food-web structure (Wells 1985; Evans and Jude 1986; Evans 1986; Scavia et al. 1986; Fahnenstiel and Scavia 1987a) has stimulated discussion of whether plankton species composition is controlled from the top (food-web effects) or from the bottom (nutrient effects) of the ecosystem (Kitchell et al. 1988). These arguments developed because Lake Michigan has experienced (1) a major shift in the composition of its planktivorous fishes, presumably caused by environmental stress (Eck and Brown 1985) and predation by stocked salmonines (Stewart et al. 1981; Kitchell and Crowder 1986), and (2) reduced P loading (DePinto et al. 1986) and in-lake P concentrations (Lesht and Rockwell 1985; Scavia et al. 1986). While the mechanisms underlying these changes have not been defined indisputably, note the following:

[1]GLERL Contribution No. 542.

(1) Total phosphorus concentration during winter–spring isothermy has decreased gradually from approximately 7.5 $\mu g \cdot L^{-1}$ in 1975 to approximately 5.5 $\mu g \cdot L^{-1}$ in 1984 (Lesht and Rockwell 1985; Scavia et al. 1986).

(2) Abundance of alewife (*Alosa pseudoharengus*), the previously dominant planktivore, had decreased by about an order of magnitude since early 1970s and in 1983–84 was at its lowest level since its years of dominance (Wells 1985). The decrease represents a factor of 3–4 between the late 1970s and the early 1980s.

(3) Zooplankton total biomass in offshore waters did not change significantly during 1975–84; however, an abrupt change in summer species composition occurred between 1982 and 1983 (Scavia et al. 1986). The previously dominant calanoid copepods (*Diaptomus ashlandi, D. minutus, D. sicilis*) were replaced first by *Daphnia pulicaria* in 1983 and then by a complex of three daphnid species (*D. pulicaria, D. galeata mendotae, D. retrocurva*) in 1984 and 1985 (Evans and Jude 1986; Scavia et al. 1986; Scavia and Fahnenstiel 1987).

(4) Current summer epilimnetic phytoplankton species composition changed from that reported for the 1970s (Schelske and Stoermer 1972; Rockwell et al. 1980; Bartone and Schelske 1982). Using the assemblage scheme of Reynolds (1984) as a convenient summary, spring and early summer remain unchanged. Initially the system is dominated by diatoms characteristic of B and C assemblages (e.g. *Melosira* spp., *Tabellaria* spp., with lesser amounts of *Asterionella formosa, Fragilaria crotonensis*) and then by phytoflagellates of assemblage Y (*Cryptomonas, Rhodomonas*) and assemblage E (*Dinobryon, Mallomonas*). In the 1970s, the phytoflagellates were succeeded by a community dominated by colonial greens and blue-greens characteristic of Reynolds' H and M assemblages (e.g. *Anabaena* spp., *Microcystis*). However, these species abruptly disappeared after 1982 and the community of 3–30 μm phytoflagellates of assemblage Y continued to dominate through the summer (Fahnenstiel and Scavia 1987a). Thus, the 1970s Lake Michigan phytoplankton seasonal succession followed B/C → E/Y → H/M, while the early 1980s pattern followed B/C → E/Y → Y.

(5) Measures of summer water clarity (Secchi depth, transmissivity, photosynthetically active radiation) appear to have improved gradually between 1975 and 1982, but also have improved more abruptly after 1982 (Scavia et al. 1986). Summer Secchi depth averaged 7.0 ± 1.3 m between 1975 and 1982, but averaged 11.1 ± 1.4 m during 1983–86 (Scavia and Fahnenstiel 1988).

We believe that alterations in phytoplankton and zooplankton species composition were caused either by (1) reduced P loading and/or (2) reduced predation on large zooplankton due to declining alewife abundance (Scavia et al. 1986; Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987a). We tested these hypotheses by constructing a model of phytoplankton–zooplankton–nutrient interactions, calibrating it to conditions (concentrations and process rates) observed in the early 1980s (Scavia et al. 1986; Fahnenstiel and Scavia 1987a, 1987b, 1987c; Scavia and Fahnenstiel 1987), and subjecting it to a range of zooplanktivory under the influence of two P-loading scenarios. The simulation analysis tests whether the model, calibrated to conditions and species mixtures of the 1980s, could reproduce the trend (backward) of species changes representative of a summer complex comprising blue-green and colonial green algae and calanoid copepods under the relatively heavy alewife predation of the mid-1970s.

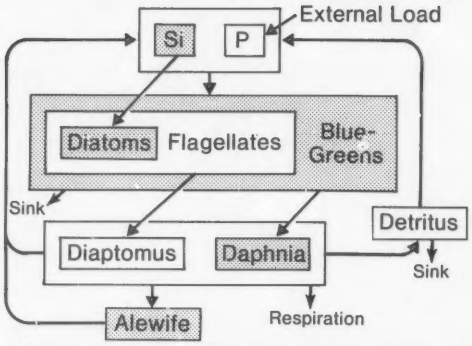

FIG. 1. Schematic of major pathways in model designed to simulate nutrient cycles and biomass flow among competing phytoplankton and zooplankton groups. Arrows represent major pathways; some are omitted for clarity. Nested boxes are used to show selective grazing pathways. For example, all algal groups are consumed by *Daphnia* whereas only flagellates and diatoms are consumed by *Diaptomus*.

Methods and Modeling Rationale

The Model

There is increasing evidence that pelagic models based on functional (e.g. Reynolds 1984) or size-grouped (e.g. Sheldon et al. 1982; Sprules et al. 1983) components offer advantages over traditional models of individual species (O'Neill et al. 1986). Changes that have occurred in Lake Michigan since the 1970s are adequately described by functional classes and we were intrigued by the possibility of exploring hypothesis on competition and predation with such an approach. Figure 1 details the functional categories of plankton used in the model.

Our approach incorporated both empirical principles correlated with size and biomass (Peters 1986) and experimentation that accounts for the specificity of functional groups expressed by parameters derived from species interactions (Lehman 1986). For example, cladoceran grazing rates and body size are related (e.g. Peters and Downing 1984); however, clear differences for cladocerans versus calanoid copepods (Knoechel and Holtby 1986a, 1986b) must be preserved. Hence, we separated zooplankton into these functional groups. Our algal functional groups were derived similarly from analysis of observed successions (Reynolds 1984) and growth rates (Sommer 1981) and from practical definitions for ecosystem models (Scavia 1979a).

In general, the model simulates competition among phytoplankton and zooplankton groups, each subject to differential grazing pressure. Algae and detritus are grazed and sedimented; algal growth is nutrient dependent. The zooplankton gain mass through food-dependent ingestion and lose mass through defecation, respiration, and fish predation. Inefficient alewife and zooplankton grazing provides input to detritus and available P pools. All ingested Si is routed directly to the detrital pool. Zooplankton P release is assumed proportional to respiration. Detrital Si settles and dissolves; detrital C and P settle and slowly decompose to available forms. Phytoplankton and zooplankton concentrations and fluxes are simulated in terms of C; associated nutrient fluxes are traced subject to a constant mass Redfield ratio (P:C = 0.024). Equations simulating these first-order processes leading to gains and losses of each modelled component are listed in Table 1. Some detailed aspects of those equations and their rationales follow.

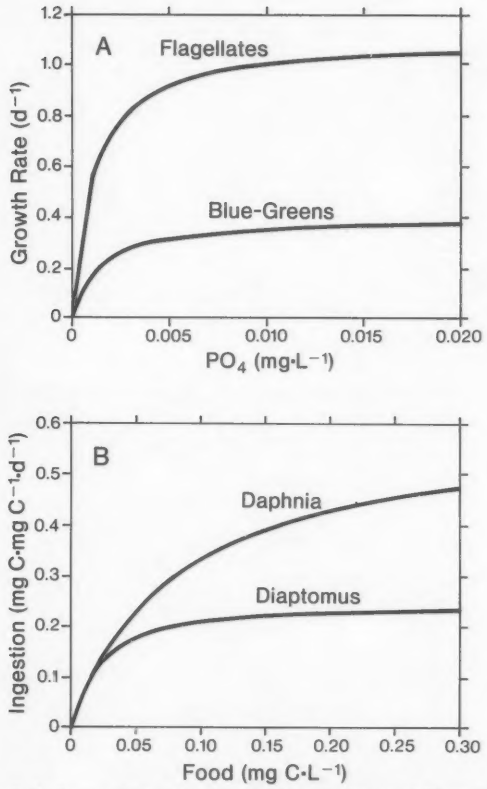

FIG. 2. Defining relationships for (A) phytoplankton and (B) zooplankton competition for resources. Both are represented as Monod responses to substrate or food concentration and the governing parameters are listed in Tables 2 and 3. For perspective, Lake Michigan summer epilimnion soluble reactive P concentrations are below 1.0 $\mu g \cdot L^{-1}$, total algal C is 20–40 $\mu g \cdot L^{-1}$, and particulate organic C is approximately 200 $\mu g \cdot L^{-1}$.

Our model of the offshore summer epilimnion assumes constant optimal light and temperature. Horizontal and vertical transport are not critical factors in the summer epilimnion (Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987b, 1987c); therefore, we ignore them.

During 1975–84, P loads decreased (DePinto et al. 1986). We included analyses for the high available-P load of the mid-1970s (0.0055 $\mu g\ P \cdot L^{-1} \cdot d^{-1}$) and the low load for 1981–82 (0.0035 $\mu g\ P \cdot L^{-1} \cdot d^{-1}$), based on estimates summarized by Rodgers and Salisbury (1981) and Lesht (1984) for a southern basin epilimnion volume of 4.28×10^{11} m^3.

Phytoplankton

Phytoplankton growth rate (μ) was modelled as a maximal rate (μ_m) scaled by the lesser of two growth-limiting factors, one for Si (diatoms only) and one for P (all groups);

$$(1) \quad \mu = \mu_m \cdot \mathrm{MIN}\{P/(K_p + P), Si/(K_{Si} + Si)\}.$$

Maximal growth rates and half-saturation constants (K) were generated from measured rate constants for species representative of our three modelled groups (Table 2). Diatoms are usually Si limited during summer both in the lake (Fahnenstiel and Scavia 1987a) and in our simulations. Competition for P between the two P-limited groups is illustrated in Fig. 2 for our kinetic constants. We used constant group-specific phytoplankton settling velocities, also drawn from several reviews (Table 2).

TABLE 1. Model equations.

State variable		Model equation
Phytoplankton	$\frac{dA}{dt}$	$= (\mu - s)A - gZ$
	A	= phytoplankton concentration
	μ	= growth rate (see text equation (1))
	s	= settling loss = sinking rate/epilimnion depth
	g	= zooplankton grazing (see text equation(2))
	Z	= zooplankton concentration
Zooplankton	$\frac{dZ}{dt}$	$= (g - r)Z - P$
	r	= respiration (see text equation(3))
	P	= fish predation = specific ration · alewife biomasses
Detrital C	$\frac{dC}{dt}$	$= B \cdot E - (d + s) \cdot C - gZ$
	C	= detrital C concentration
	B	= particulate fraction of unassimilated food
	E	= zooplankton egestion = (1 − assimilation efficiency)·ingestion
	d	= mineralization rate
Available P	$\frac{dP}{dt}$	$= PC \cdot [\Sigma rZ + (1 - B) \cdot E - \Sigma \mu A + d \cdot C]$
	P	= available P concentration
	ΣrZ	= zooplankton respiration
	$\Sigma \mu A$	= phytoplankton production
	PC	= P:C ratio
Available Si	$\frac{dSi}{dt}$	$= d_s \cdot S_d - SC \cdot \mu_d A_d$ *S
	Si	= available Si concentration
	S_d	= detrital Si concentration
	d_s	= dissolution rate
	μ_d	= diatom growth rate
	A_d	= diatom concentration
	SC	= Si:C ratio
Detrital Si	$\frac{dS_d}{dt}$	$= SC \cdot E_d - (d_s + s) \cdot S_d$
	E_d	= egested silica

Zooplankton

Zooplankton grazing rates (g) are based on saturation kinetics for feeding and the "effective food concentration" (EFC) construct of Vanderploeg et al. (1984):

$$(2) \quad g = g_m \cdot \mathrm{EFC}/(K + \mathrm{EFC})$$

where g_m is the maximal weight-specific ingestion rate and K is the half-saturation constant. The EFC is defined as $\Sigma W'_i X_i$,

TABLE 2. Properties of modelled algal groups. Each set of values from the literature represents the mean, standard deviation (in parentheses), and number of species included in the mean.

Parameter	Diatoms	Flagellates	Blue-greens	Source
Maximal growth	1.09(0.42)6	0.82(0.01)2	0.79(0.46)10	Reynolds 1983
rate (d^{-1})	0.73(0.23)28	—	0.57(0.22)5	Tilman et al. 1982
	1.47(0.37)17	0.79(0.11)4[a]	—	Sommer 1983
		1.18(0.27)6	—	Reynolds et al. 1982
	0.8–1.8	1.0–1.4	<0.7	Reynolds 1984
	1.5	*1.1*	*0.4*	Used in this study
Monod constants				
$\mu g\ P \cdot L^{-1}$	3.0(5.0)11	—	0.8(0.4)4	Tilman et al. 1982
	2.5	*1.0*	*1.3*	Used in this study
$\mu g\ Si \cdot L^{-1}$	0.11(.014)25	—	—	Tilman et al. 1982
	0.10	*0*	*0*	Used in this study
Sinking rates	1.2(0.7)2	0.01(0.004)3	—	Fahnenstiel and Scavia 1987a
($m \cdot d^{-1}$)	1.0(0.4)7	0.0	0.04(0.004)	Sommer 1984
	0.5(0.3)9	0.0	—	Reynolds and Wiseman 1982
	1.0(1.3)37[b]	—	—	Wetzel 1983
	0.8	*0.05*	*0.05*	Used in this study

[a]Values are likely low due to mechanical hampering.
[b]Used minimal and maximal values to calculate averages when ranges were reported.

TABLE 3. Properties of modelled zooplankton groups.

Parameter	*Daphnia*	*Diaptomus*	Source
Maximal ingestion	0.71	0.2	Muck and Lampert 1984
rate	0.86	—	Olsen et al. 1986
($mg\ C \cdot mg\ C^{-1} \cdot d^{-1}$)	0.61[a]	—	Peters and Downing 1984
	0.84[b]	—	Peters and Downing 1984
	0.2–0.3	—	Porter et al. 1982
	—	0.2	Vanderploeg et al. 1984
	—[c]	—[c]	Knoechel and Holtby 1986a
	—[c]	—[c]	Knoechel and Holtby 1986b
	0.60	*0.25*	Used in this study
Half-saturation	0.12	0.01–0.05	Muck and Lampert 1984
constant	0.05–0.10	—	Porter et al. 1982
($mg\ C \cdot L^{-1}$)	—	0.02	Vanderploeg et al. 1984
	0.08	*0.02*	Used in this study

[a]From body weight regression assuming 10 μg dry weight and 1 mg $C \cdot L^{-1}$ food.
[b]From body weight regression assuming 30 μg dry weight and 5 mg $C \cdot L^{-1}$ food.
[c]Clearance rates on algae and bacteria were 5–10 times lower for calanoid copepods of the same size as comparable cladocerans in the cited report.

where W'_i is the selectivity coefficient for food source i with concentration X_i (Vanderploeg and Scavia 1979).

While the mathematical form of the relationshp between zooplankton ingestion and food concentration is arguable (Mullin et al. 1975; Porter et al. 1982), we use equation (2) which is similar to the Monod expression for phytoplankton growth. Maximal weight-specific ingestion rates and half-saturation constants, based largely on the works of Porter et al. (1982), Muck and Lampert (1984), Peters (1984), Peters and Downing (1984), Vanderploeg et al. (1984), and Olsen et al. (1986), are listed in Table 3 and are illustrated in Fig. 2.

Equation (2) estimates ingestion of all prey by a given predator. Loss rates for an individual prey item i are estimated from the product of equation (2) and the weighted diet portion attributable to prey i, $W'_i X_i / \Sigma W'_i X_i$. The rationale for selectivity coefficient (W'_i) values acknowledges that the phytoflagellates (3–30 μm) are within the size range of high preference for both calanoid copepods (Vanderploeg 1981; Vanderploeg et al. 1984) and *Daphnia* (Gliwicz 1980; Knisely and Geller 1986); thus, they are a most preferred prey ($W'_i = 1$). Both calanoids and *Daphnia* can ingest some diatoms (Knisely and Geller 1986; H. A. Vanderploeg, GLERL/NOAA, pers. comm.) and were thus assigned W'_i values of 0.5. The dominant calanoids (*Diaptomus* spp.) are highly selective against filamentous prey (Vanderploeg 1981; Hartmann 1985); *Daphnia* are not (Porter et al. 1982; Richman and Dodson 1983). We assigned a W'_i of 0.5 for colonial greens and blue-greens grazed by *Daphnia* and 0.1 for grazing by the calanoids.

Assimilation efficiencies were assumed to be 50% on all algal foods (48%, Comita 1972; 75%, Porter et al. 1982; 50%, Peters 1983; 50%, Parsons et al. 1984, 38%, Peters and Downing 1984) and 10% on detritus. Because zooplankton respiration rate is generally higher for well-fed animals than for starved ones (Porter et al. 1982; Lampert 1984; Parsons et al. 1984), we estimated respiration (r) as proportional to the ingestion function of equation (2):

(3) $r = r_1 + r_2 \cdot \mathrm{EFC}/(K + \mathrm{EFC})$.

Maximal respiration rates generally fall in the range of 0.1–0.4 mg C·mg $C^{-1}\cdot d^{-1}$ (Porter et al. 1982; Richman and Dodson 1983; Lampert 1984; Parsons et al. 1984; Lynch et al. 1986) with rates for well-fed animals typically 1.2–3.0 times higher than starved ones (Porter et al. 1982; Lampert 1984; Parsons et al. 1984). We use maximal rates of $0.25\cdot d^{-1}$ for *Daphnia* and $0.11\cdot d^{-1}$ for *Diaptomus*. The difference between *Daphnia* and *Diaptomus* respiration rates reflects different behavioral and feeding modes (Porter et al. 1982) as well as observations of body C loss under low food conditions (Muck and Lampert 1984).

Fish Predation

The functional underpinnings of alewife predation are not well understood. Nonetheless, we estimate, in a general way, the magnitude of alewife predation with a time-tested (Rice and Cochran 1984; Bartell et al. 1986) approach based on a bioenergetics model of alewife population structure and diets observed during 1976. The original energetics parameters were those developed by Stewart (1980) and modified by Stewart and Binkowski (1986).

From June through August of 1976, lake-wide mean feeding rates were estimated as 1.76×10^7 kg of zooplankton prey consumed per day. With summer alewife biomass of 7.6×10^7 kg (Stewart 1980) the mean daily ration becomes $(1.76/7.6) = 0.23\cdot d^{-1}$, which is higher than that for other fishes, due to high feeding rates by juveniles (Stewart 1980; Kitchell 1983). We held this ration constant during simulations and varied alewife biomass to mimic its changing impact over time (see below).

Alewife prey selection was based on studies of prey preferences, foraging behavior, and optimal foraging theory (Morsell and Norden 1968; Webb and McComish 1974; Rhodes and McComish 1975; Janssen 1978; Janssen and Brandt 1980; Wells 1980; Crowder et al. 1981). The model treats alewife as a size-selective predator with preferences of major prey ordered as macroinvertebrates (*Mysis* and *Pontoporeia*) > cladocerans >> copepods. A biomass model such as ours can only include diet ontogeny by weighting selectivity coefficients. These coefficients can be derived as estimates of prey preference from forage ratios based on the fraction of prey *i* biomass in the summer diet (r_i) divided by the portion of prey *i* biomass in the available plankton (p_i) (Vanderploeg and Scavia 1979). From field studies conducted during the mid-1970s (Stewart 1980), diets of alewife (r_i) were 30% copepods, 20% cladocerans, and 50% hypolimnetic *Mysis* and *Pontoporeia*. During these survey years, *Daphnia* comprised only about 10–15% of the total zooplankton biomass (net hauls through top 40 m; Scavia et al. 1986); the remainder was dominated by calanoid copepods. Sufficient data are not available to estimate accurately the biomass concentration of *Mysis* and *Pontoporeia* available to alewife during those years; however, we can make certain estimates. *Pontoporeia* are likely not available to offshore (100-m depth contour) alewife during summer because the invertebrate is benthic and the alewife planktonic. Thus, the major macroinvertebrate prey is likely to be *Mysis*. Sell (1982) estimated its abundance to vary between 0.1 and 1.11 g dry weight·m^{-2} in Lake Michigan. Assuming a migratory behavior that places *Mysis* within a 15-m band available to alewife for about 6 h per day (Bowers and Grossenickle 1978) and a C : dry weight ratio of 0.5 yields a C concentration averaging 5.1 $\mu g\cdot L^{-1}$. This value is not unlike that for *Daphnia* during the mid-1970s (Scavia et al. 1986). If we assume that macroinvertebrate abundance ranged between being equal to the cladocerans and twice the cladocerans, then relative p_i estimates are 85–90, 5, and 5–10% for copepods, cladocerans, and the hypolimnetic invertebrates, respectively. Setting the largest W'_{ij} to 1.0 and scaling the rest for convenience results in values of 0.03–0.07, 0.4–0.8, and 1.0 for copepods, cladocerans, and *Mysis*; we used 0.05, 0.5, and 1.0 in the model.

Simulated zooplankton loss rates were calculated from the product of alewife biomass, daily ration, and diet proportion ($W'_i X_i / \Sigma W'_i X_i$), where X_i is the concentration of prey *i*. We assume a constant pool of hypolimnetic prey equivalent to 5.0 $\mu g\ C\cdot L^{-1}$.

TABLE 4. Lake Michigan summer epilimnion conditions measured in 1983–84 (Data from Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987a, 1987b, 1987c; Scavia et al. 1986).

Property	Concentration or rate
Algal growth rates	$0.1–0.4\ d^{-1}$
Algal production	$12.9 \pm 2.2\ \mu g\ C\cdot L^{-1}\cdot d^{-1}$
Algal biomass	20–40 $\mu g\ C\cdot L^{-1}$
	Flagellate dominance
Algal sedimentation	$0.02–0.45\ \mu g\ C\cdot L^{-1}\cdot d^{-1}$
Zooplankton grazing	$8.7 \pm 2.5\ \mu g\ C\cdot L^{-1}\cdot d^{-1}$
Zooplankton biomass	20–35 $\mu g\ C\cdot L^{-1}$
	Daphnia dominance

Nutrient Cycles

Available P concentration was determined by a balance between phytoplankton uptake, detritus mineralization ($0.01\cdot d^{-1}$), and alewife and zooplankton release. A portion of food ingested but not assimilated by alewife and zooplankton was shunted to detritus, with the remaining portion shunted immediately to available nutrient pools. We assumed that 50% of egested P becomes available immediately to algae on the time scale of our simulations. Available Si concentration was determined by the balance between diatom uptake and detrital Si dissolution. In addition, all Si ingested by zooplankton was immediately placed in the detrital Si pool.

Calibration

For the calibration data set, we relied primarily on measurements made during the Lake Michigan Ecosystem Experiment of 1983–84 (Scavia et al. 1986; Scavia and Fahnenstiel 1987; Fahnenstiel and Scavia 1987a, 1987b, 1987c). The field experiment was designed to measure rates of phytoplankton growth and sedimentation and zooplankton grazing, as well as temporal and spatial variation of nutrients and phytoplankton and zooplankton species composition. The current state of southeastern Lake Michigan, based on the 1983–84 field seasons, is summarized in Table 4. For cases where comparable process rates were not determined empirically for Lake Michigan, we used published values from other lakes to establish bounds on model expectations.

Calibration consisted of varying coefficient values within ranges expected for Lake Michigan phytoplankton and zooplankton assemblages (Tables 2 and 3) until the model reproduced both measured state variables *and* process rates measured in the 1980s. Because coupled nonlinear differential equations such as the ones used here do not have unique solutions, it is possible to arrive at similar state-variable simulations with very

different sets of coefficient values, and thus, rates (Scavia 1979b, 1980, 1983). Therefore, requiring accurate reproduction of both state variables *and* process rates should minimize compensating rate-process errors and produce a reasonable model caricature of Lake Michigan ecosystem dynamics.

The nine differential, mass-balance equations describing ecosystem dynamics (Table 1) were solved numerically using a forward-difference Euler scheme with a time step of 2.4 h. Model output was stored every 25 d and simulations were run until competition results were clearly defined (usually 300 d, see below).

Alewife Impact Scenario

Lake Michigan alewife were first reported in 1949 (Smith 1970). The population expanded exponentially until a major overwinter mortality in 1966–67 and thereafter remained generally at less than half its peak abundance. Recently, alewife abundance has further decreased (Wells 1985). Although stock assessments and estimates of local variability exist, no unequivocal quantitative measures are available for lake-wide alewife abundance (Eck and Brown 1985). The alewife's distribution is highly patchy; they school, migrate vertically, and generally aggregate (vertically and horizontally) near the edges of the metalimnion during summer (Crowder et al. 1981). Abundance peaks near the 20 to 30-m depth contour and decreases exponentially offshore toward the region of the 100-m contour (Brandt 1980), the area we attempt to simulate. Hence, it is difficult to determine the absolute effective biomass impacting the epilimnion in our region of interest.

Initial model analyses demonstrated that we could not reproduce plankton dynamics observed at the 100-m contour under the grazing pressure imposed using the high lake-wide average alewife biomass estimates of the 1970s (75 000 Mt; Wells and Hatch 1985). For alewife biomasses above 15 000 Mt, simulated zooplankton and phytoplankton relative species composition remained constant; but total zooplankton biomass was reduced to unrealistically low levels, phytoplankton biomass increased to unrealistically high levels, and process rates were far below those observed. Given these results and the uncertainties on biomass impact estimates discussed above, we restricted our analyses to a range of lake-wide alewife biomasses from 0 to 15 000 Mt and explored species changes along the relative alewife biomass gradient.

Assuming an equal north–south distribution, an alewife C : wet weight ratio of 0.15 and a strictly epilimnetic existence, this range is equivalent to 0–2.64 $\mu g\ C \cdot L^{-1}$. Because it is difficult to assign an alewife biomass level to a particular set of years, we simulated the entire zooplanktivory gradient for each of the two P-loading scenarios: one reflecting conditions of the mid-1970s ($0.0055\ \mu g\ P \cdot L^{-1} \cdot d^{-1}$) and one of the early 1980s ($0.0035\ \mu g\ P \cdot L^{-1} \cdot d^{-1}$).

Uncertainty Analysis

We recognize the difficulty in justifying any one set of coefficient values used in the model, even though they fall within observed ranges and produce internally consistent and reasonably faithful reproduction of measured state variable and process rates. To explore the robustness of our simulations, we performed Monte Carlo analyses including 14 model coefficients as the random variables. We generated 300 sets of random and independent coefficient values, drawn from a 14-variate normal distribution (International Mathematical and Statistical Libraries subroutine GGNSM). Each coefficient was assigned a mean value equal to the value used in the deterministic simulation and a standard deviation equal to 10% of its mean. While 10% may not represent the true extent of coefficient variability, it is sufficient to use here to evaluate overall model sensitivity. A simulation was run for each set of coefficient values resulting in 300 time series for each state variable; we analyzed results at 300 d into the simulation, the same as for the deterministic model. Monte Carlo results were similar when more than 300 cases were used.

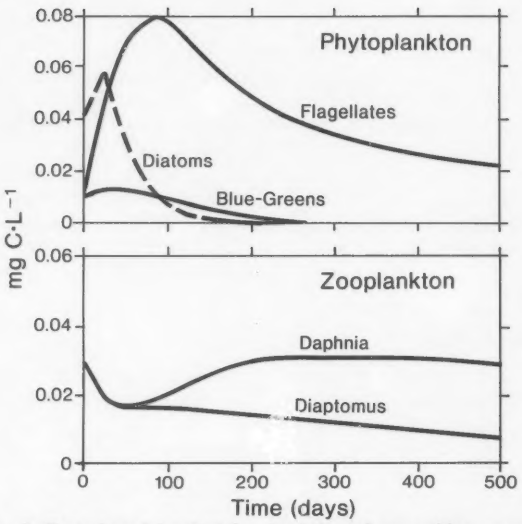

FIG. 3. Typical model output for simulation of competition among phytoplankton and zooplankton groups under low alewife abundance and low P load. Each simulation displays a transient response followed by slow development toward steady state. We use results at 300 d into the simulation throughout the analysis (see text).

Results

Deterministic Simulations

As expected for solution of differential equations with constant inputs, each simulation (Fig. 3) began with a transient response followed by a more stable period approaching steady state. While transient responses are influenced strongly by initial conditions, steady-state responses are independent of those starting values, depending only on the nature of the equations and values of the coefficients and inputs. Because very different transient responses can be effected with different starting values, and because we do not know starting values, we used simulation results clearly beyond the influence of the initial conditions (e.g. after 200 simulation days, Fig. 3).

In theory, as well as in continuous-culture competition experiments (e.g. Tilman 1982) with only one potentially limiting nutrient, one species will exist at steady state. While our analyses have two potentially limiting nutrients (P, Si), heavy sedimentary losses inflicted on the Si-limited diatoms essentially eliminate them from the summer competition. As a result, two species (modelled flagellate and blue-green groups in this case) are always left to compete for P and the model always predicts complete dominance by one of the two groups at steady state (usually after 400–500 simulation days). However, Lake Michigan is not a continuous culture and steady conditions rarely persist. It is not realistic therefore to expect

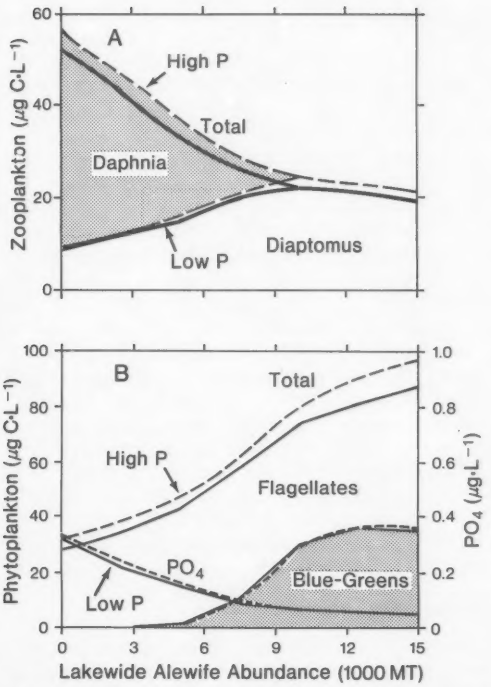

FIG. 4. (A) Simulated zooplankton biomass (total and groups) as a function of P load (high, broken line; low, solid line) and alewife abundance. The relative contribution of *Daphnia* to total biomass diminishes as alewife abundance increases. (B) Simulated total phytoplankton (total and groups) and PO_4 concentration as a function of P load (high, broken line; low, solid line) and alewife abundance. Flagellate concentration remains relatively constant, while blue-greens increase at higher alewife abundances.

the lake ever to be at steady state nor to run the model until those conditions prevail. We use 300 d into the simulation for our comparisons of alewife and P-load impacts. This is a reasonable compromise because, at this point in all simulations, the model is no longer under the influence of the transient response and the outcome of the competition is clear. Selecting time points further toward model steady state will only influence the abundance of total plankton; the overall trends in composition are unaffected.

Simulation days are not to be confused with actual days in the sense of time to reach steady state. The analysis does not suggest a time scale to steady state on the order of 1 yr (300 d) because the duration of the transient response is controlled by the extent to which the initial conditions deviate from steady state. For example, if we chose initial conditions exactly equal to the steady-state response, there would have been no transients.

Results of model runs for a range of zooplanktivory are summarized as response-surface plots (Fig. 4–7) of plankton abundances and process rates versus alewife abundance for the two P-load scenarios. Total zooplankton biomass is slightly higher under the higher P load for all levels of alewife abundance; however, the overall impact of alewife is much larger (Fig. 4A). As we increase alewife biomass, total zooplankton biomass decreases; more importantly, composition changes from *Daphnia* dominance to *Diaptomus* dominance.

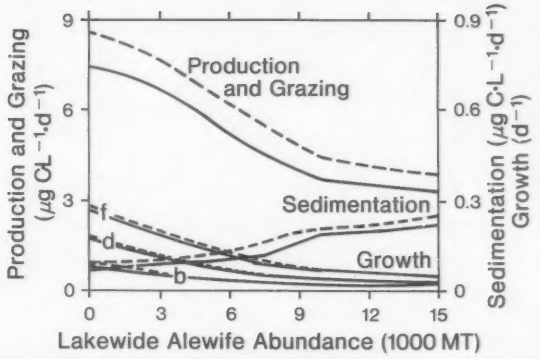

FIG. 5. Simulated phytoplankton production (≈ zooplankton grazing), sedimentation, and algal growth rates (f = flagellates, b = blue-greens, d = diatoms) as a function of P load (high, broken line; low, solid line) and alewife abundance. As alewife abundance increases, algal growth rates decrease and the importance of sinking, relative to grazing, increases.

Total phytoplankton biomass increases with increasing alewife biomass over this range of alewife abundance (Fig. 4B); the responses at both P loads are similar. Phytoplankton composition changes as well. The flagellate dominance under low alewife levels is replaced by an assemblage composed of flagellates (approximately 60%) and blue-greens (approximately 40%) under high alewife levels. Available P concentrations decrease at higher alewife levels (Fig. 4B), with the implication that more P is tied up in phytoplankton biomass.

As alewife abundance increases and P concentrations decrease, primary production and phytoplankton growth rates both decrease (Fig. 5). Although phytoplankton biomass increases, the lower growth rates controlled by lower P concentrations and the shift to slower growing blue-greens yield lower epilimnetic primary production rates. Growth rates decrease sevenfold over the full range of zooplanktivory; primary production decreases only twofold. Sedimentation loss rates increase from about 0.10 μg $C \cdot L^{-1} \cdot d^{-1}$ with no alewife predation to about 0.24 μg $C \cdot L^{-1} \cdot d^{-1}$ under maximal alewife biomass at the higher P load. Sedimentation increases from 0.07 to 0.22 μg $C \cdot L^{-1} \cdot d^{-1}$ for the low P load.

Because sedimentary losses are small during summer, model net primary production is nearly balanced by zooplankton grazing. Calculated ingestion minus defecation and respiration yield model estimates of zooplankton secondary production (Fig. 6A). Total zooplankton production increases as alewife abundance increases, but appears to level off at biomass levels above 5000 Mt. *Daphnia* production decreases as their contribution to total zooplankton biomass decreases. At low levels of zooplanktivory, *Diaptomus* is actually a negative contributor to secondary production (Fig. 6A). Under these conditions, *Daphnia* flourish and drive phytoplankton concentrations below the level that allow *Diaptomus* to survive. This result can be seen most readily in terms of relative production to biomass ratios (net growth) for the two groups at different levels of zooplanktivory (Fig. 6B). It is not until alewife abundance reaches approximately 5000 Mt and total phytoplankton C reaches approximately 40 μg $C \cdot L^{-1}$ that *Diaptomus* ingestion rates exceed egestion and respiration losses. Beyond that, they grow but still slowly relative to *Daphnia*.

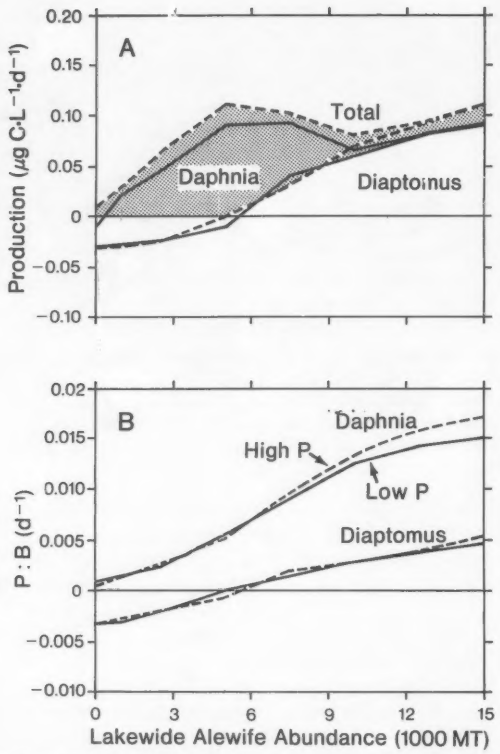

FIG. 6. Simulated zooplankton (A) secondary production and (B) production to biomass ratios as a function of P load (high, broken line; low, solid line) and alewife abundance. This suggests that during summer in Lake Michigan's epilimnion, *Daphnia* is always the superior competitor and only selective losses can shift the dominance toward *Diaptomus*.

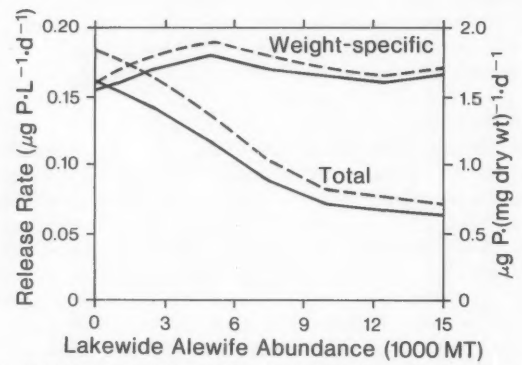

FIG. 7. Total and weight-specific zooplankton P regeneration rates as a function of P load and alewife abundance.

Phosphorus recycling by zooplankton is the major P supply supporting phytoplankton production. The decreased supply rate at higher alewife abundance (Fig. 7) reflects mostly decreased total zooplankton biomass (Fig. 4A). As excretion rates are tied to group-specific rates of grazing and respiration, however, weight-specific excretion rates also varied as the phytoplankton and zooplankton assemblage compositions changed (Fig. 7).

Summary of Model Results

In general, several ecosystem properties show dramatic changes along the predation gradient, whereas only small changes are evident when comparing results from the high and low P-load scenarios. As alewife abundance increases, (1) total zooplankton biomass decreases and the composition shifts from *Daphnia* dominance to *Diaptomus* dominance (Fig. 4A), (2) total phytoplankton biomass increases and the composition changes from dominance by flagellates to a mixture of flagellates and blue-greens (Fig. 4B), (3) primary production and zooplankton ingestion rates decrease and are nearly equal to each other (Fig. 5), (4) phytoplankton growth rates decrease more dramatically than production rates, (5) sedimentation rates increase but are never an important component of phytoplankton dynamics, (6) P regeneration by zooplankton (Fig. 7) and available P concentrations (Fig. 4B) decrease, and (7) zooplankton secondary production and growth rates increase (Fig. 6), with *Diaptomus* growth rates varying from negative to positive though always below *Daphnia* growth rates. These simulated changes are similar to those described for Lake Michigan during changing abundances of alewife. Thus, the model appears to reproduce the cascading influence of zooplanktivory on the Lake Michigan food web.

Uncertainty Analysis

For error analysis, we chose model coefficients considered to be most uncertain, most critical to the analysis, or both; phytoplankton maximal growth rates and half-saturation constants for P-limited growth; zooplankton maximal ingestion rates, half-saturation constants for ingestion, and respiration rates; and the selectivity coefficients for zooplankton on bluegreens and for alewife on zooplankton. We report medians (Fig. 8) for the Monte Carlo results because the distributions of model output were strongly skewed, as was the case in analysis of a similar model (Scavia et al. 1981). While the Monte Carlo medians differ from the deterministic projections described above (Fig. 4), the overall trends and shifts in plankton composition are the same. In 19 of the 20 cases (i.e. 4 state variables at 5 alewife abundances), the deterministic simulations fall within 12.5% of the medians. (Those ±12.5% limits are shown in Fig. 8). We interpret the fact that the deterministic solutions are similar to the stochastic medians to mean that the deterministic simulations we presented above are rather robust.

However, to what extent are the deterministic simulations realistic representations of Lake Michigan ecosystem dynamics as opposed to simple mathematical exercises?

Discussion

Comparison with the 1980s

Our first task is to evaluate whether model results under conditions of low alewife abundance are similar to those observed during 1983–84 (Table 4). Lake Michigan summer phytoplankton concentrations ranged between 20 and 40 μg $C \cdot L^{-1}$ (Scavia and Fahnenstiel 1987), averaging over 80% as phytoflagellates (range = 70–90%) (Fahnenstiel and Scavia 1987a). These conditions are reproduced by the model for alewife biomass levels below 5000–7500 Mt. Summer zooplankton concentrations ranged between 20 and 35 μg $C \cdot L^{-1}$ (Scavia and Fahnenstiel 1987), with C assumed to be 50% of dry weight, although there has been little observable systematic variation over the past 10 yr (mean = 40 μg $C \cdot L^{-1}$,

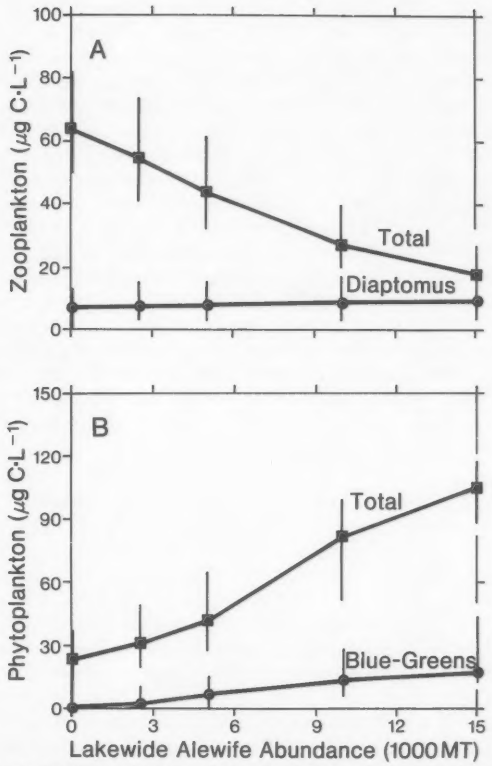

FIG. 8. Medians of Monte Carlo simulations of phytoplankton and zooplankton concentration as functions of alewife abundance at low P load. Deterministic simulations (see Fig. 4) generally fall within ±12.5% of the medians, shown here as error bars.

SD = 10.8; Scavia et al. 1986). During the early 1980s, however, *Daphnia* contributed about 70–90% to crustacean biomass. These conditions are reproduced by the model for alewife levels between 2500 and 5000 Mt.

Models can reproduce concentrations of ecosystem variables, yet severely under- and overestimate rates of material flow (Scavia 1980). As an example, summer phytoplankton dynamics are controlled by a balance between growth and grazing loss. Growth is controlled by the P supply, which is largely determined by zooplankton regeneration. Because P regeneration in the model (and likely in nature) is tightly coupled to zooplankton ingestion, both phytoplankton growth and loss rates are influenced strongly by zooplankton. It is easy to imagine situations where algal growth and zooplankton grazing both could be low or high and the resulting rate of algal biomass change be the same. Thus, we could get the right results (state-variable concentrations) for the wrong reasons (compensating rate processes). So, we must compare modelled and measured rates.

Measured production and loss rates from the 1983–84 field seasons are comparable in magnitude for much of the summer, although some unaccountable algal loss may have been evident (Scavia and Fahnenstiel 1987). Because the only loss included in the model (other than grazing) was sedimentation and because sedimentation rates were low (both in the model, 0.07–0.24 μg $C \cdot L^{-1} \cdot d^{-1}$, and in the field experiments, 0.02–0.45 μg $C \cdot L^{-1} \cdot d^{-1}$; Scavia and Fahnenstiel 1987), model estimates of phytoplankton net production and zooplankton grazing are in close balance. Net epilimnetic production averaged 12.9 μg $C \cdot L^{-1} \cdot d^{-1}$ (SE = 2.2, N = 8) and zooplankton grazing averaged 8.7 μg $C \cdot L^{-1} \cdot d^{-1}$ (SE = 2.5, N = 5) during 1983–84. These primary production estimates are somewhat higher than those simulated by the model, but the grazing rate estimates are similar to model results reproduced with alewife abundances less than 5000 Mt. Estimates of algal summer growth rates typically fall between 0.1 and 0.4·d^{-1} (Fahnenstiel and Scavia 1987b), consistent with predictions with alewife biomass below 5000 Mt.

Less is known specifically about Lake Michigan zooplankton processes. While our estimates of phytoplankton ingestion are similar to those determined in the field, we must rely on estimates from other environments for comparisons of secondary production, zooplankton growth, and P excretion. Secondary production is relatively constant (0.1 μg $C \cdot L^{-1} \cdot d^{-1}$) for model evaluations above 2500 Mt of alewife. Primary production varies between 3 and 6 μg $C \cdot L^{-1} \cdot d^{-1}$ over this range, yielding 1.7–3.3% transfer efficiencies for these herbivores. While these values appear somewhat low (5–15% might be expected, Wetzel 1983), they are within the ranges reported across several freshwater environments (13–15%, Blazka et al. 1980; 0.1–27.4%, Brylinsky 1980; 1.7–14%, Winberg 1980).

Production to biomass ratios (*P:B*) are another way to evaluate the validity of model calculations. Our daily *P:B* ratios range from −0.002 to +0.003·d^{-1} for *Diaptomus* and from 0.003 to 0.013·d^{-1} for *Daphnia* between alewife abundances of 2500 and 10 000 Mt. These values, like the transfer efficiencies above, are similar to those at the lower end of recent summaries (0.003–0.30, Morgan et al. 1980; 0.001–0.17, Winberg 1980). Also, our simulated difference between *Daphnia* and *Diaptomus* is consistent with the generalization that *P:B* ratios for cladocerans are higher than those for calanoid copepods, even at cold temperatures (see Fig. 6.4e in Morgan et al. 1980).

Model analysis suggests that P recycling by zooplankton decreased with higher alewife abundance; however, because weight-specific rates were less variable, the trend was actually a reflection of decreased zooplankton abundance. Weight-specific rates varied between 1.6 and 1.9 μg P·mg dry $wt^{-1} \cdot d^{-1}$ (assuming 0.5 g C·g dry $weight^{-1}$). These values are well within the range expected for freshwater crustaceans (Lehman 1980; Wetzel 1983).

The above sets of comparisons between model and experimental results suggest that the model reasonably represents pelagic ecosystem structure and function in Lake Michigan. It reproduces observed state-variable concentrations and process rates using model equations and coefficients drawn from empirical studies. While the model does not contain the richness of the natural environment with respect to potential responses to variations in driving forces, it can be considered a caricature of the lake. In this respect, the simplified version of nature can be perturbed numerically with its responses being indications of the directions nature may take.

Top-down control

The above comparisons demonstrate that model simulations, with alewife equivalent to lake-wide abundance of 2500–5000 Mt, are consistent with measured ecosystem components and processes during the 1980s. The model also reproduced plankton composition changes consistent with those observed over

the period 1975–84 (Fig. 4). The simulated changes to the 1970s composition occurred for alewife equivalent to lake-wide abundance of 10 000 – 12 500 Mt, a three- to five-fold increase over the estimates used for simulation of the 1980s. While the absolute alewife abundances that we used are lower than current lake-wide assessments (see above), estimates from those assessments did change by about a factor of 4 during the period 1975–84 (Wells 1985). Thus, the model reproduces the conditions of the 1980s *and* the observed transitions over the previous decade within the confines of observed relative changes in alewife abundance. This result was accomplished with model constructs and coefficients drawn from empirical evidence (Tables 1–3). Because changes in species make-up was reproduced by varying alewife abundance only, we conclude that the transitions of the 1975–84 period were likely driven mainly from the top of the food web.

The nature of this top-down control can be dissected further by inspecting other model calculations. Analysis of *Daphnia* and *Diaptomus* growth rates (Fig. 6) under the conditions prescribed by modelled ingestion, egestion, and respiration (Table 3, above) suggests that Lake Michigan has always been a "*Daphnia* lake" with regard to competition for food; *Diaptomus* growth rates are always lower than those for *Daphnia*. This suggestion places Lake Michigan within the region of *Daphnia* dominance suggested by Richman and Dodson (1983) in their graphical model of *Daphnia*–calanoid competition along food quality/quantity gradients. We suggest that the transition from the 1970s to 1980s represents movement within that region rather than across its boundaries. The phytoplankton composition of the 1970s in Lake Michigan was not as low quality, for example, as that of inner Green Bay (Richman and Dodson 1983) nor of Lake Washington in its eutrophic state (Edmondson and Litt 1982). During the 1970s, Lake Michigan summer epilimnion chlorophyll concentrations were typically 1 $\mu g \cdot L^{-1}$ or less (Scavia et al. 1986) and blue-green and colonial green algae comprised 50–70% of total algal mass (Fahnenstiel and Scavia 1987a). This biomass is equivalent to approximately 20 $\mu g\ C \cdot L^{-1}$, calculated from data presented by Bartone and Schelske (1982). In contrast, summer chlorophyll concentrations were between 4 and 24 $\mu g \cdot L^{-1}$ in Lake Washington during the period (1965–75; Edmondson and Lehman 1981) when *Daphnia*'s major predator, *Neomysis mercedis*, was absent but *Daphnia*'s populations were limited by elevated blue-green abundance (Edmondson and Litt 1982). Total blue-green abundance during the summers of those years was likely between 400 and 800 $\mu g\ C \cdot L^{-1}$ (applying 1 g wet weight$\cdot cm^{-3}$ and a C : wet weight ratio of 0.1 to phytoplankton data reported in Edmonson and Litt 1982). In southern Green Bay, Lake Michigan, where blue-green algae dominate during summer and *Daphnia* appear to have difficulty competing with *Diaptomus* (Richman and Dodson 1983), summer average chlorophyll concentrations range between 20 and 80 $\mu g \cdot L^{-1}$ (Auer et al. 1986). These conditions are also more extreme than those found even in the 1970s in southern Lake Michigan. While excessive blue-green filament populations are apparently detrimental to *Daphnia*, we suggest that moderate concentrations are not as harmful; it is only when selective alewife predation increased *Daphnia* mortality compared with *Diaptomus* that the latter could succeed.

Why did Lake Michigan blue-greens decrease at lower alewife abudances? It is clear from the empirical evidence input to the model and from the literature (Table 2; Sommer 1981) that blue-green filaments have lower intrinsic growth rates than phytoflagellates (Fig. 5). Because both groups are subject to minimal sinking losses, it is only when grazing imparts selective losses against the flagellates that blue-greens outcompete them. During periods of *Diaptomus* dominance, the calanoids activity select against collecting the filaments and select for the competing flagellates. During periods of *Daphnia* dominance, grazing losses are more evenly distributed and the filaments lose ground to the faster growing flagellates. While the wider trophic niche (Infante and Edmondson 1985) for *Daphnia* could be either a disadvantage (Porter and Orcutt 1980; Infante and Abella 1985) or an advantage (Infante and Edmondson 1985), it is a disadvantage for the blue-green filaments, unless the filaments become so abundant that they interfere seriously with *Daphnia*'s food gathering (Gliwicz 1980) or increase its metabolic losses (Porter and McDonough 1984). As discussed above, with moderate filament population sizes in Lake Michigan, the cladoceran seems to effectively reduce the blue-green's success by increasing the alga's mortality rate over that imposed by the diaptomid community.

The future: potential impact of new invertebrate predator

A potential epilimnetic invertebrate predator, *Bythotrephes cederstroemi*, has been recently reported in Lakes Ontario, Erie, Huron, and Michigan (Bur et al. 1986; Lange and Cap 1986; G. L. Fahnenstiel and H. A. Vanderploeg, GLERL/NOAA, per. comm.). Invertebrate predation can have important consequences for plankton community structure. To evaluate potential invertebrate impacts for Lake Michigan, we added an invertebrate predator to the model. We used model coefficient values characteristic of *Bythotrephes*: a constant clearance rate (4 L·mg dry weight$^{-1}\cdot d^{-1}$; H. A. Vanderploeg, unpubl. data) and a maximal respiration rate (0.2 mg C·mg dry weight$^{-1}\cdot d^{-1}$) based on N and P excretion rates (0.9 μg N·mg dry weight$^{-1}\cdot h^{-1}$, 0.2 μg P·mg dry weight$^{-1}\cdot h^{-1}$; W. S. Gardner, GLERL/NOAA, unpubl. data) and assumed elemental mass ratios typical of zooplankton (C : dry weight = 0.5, C:N = 4.0, C:P = 75; Parsons et al. 1984). Respiration half-saturation constant was set to 0.12 mg $C \cdot L^{-1}$ and we assumed that *Bythotrephes* would be a favored fish prey ($W'_i = 1.0$). If *Bythotrephes* prefers *Daphnia* over *Diaptomus*, as preliminary data suggest (H. A. Vanderploeg, unpubl. data), the model predicts that increased *Bythotrephes* abundance will cause Lake Michigan's plankton to return to a community similar to that of the 1970s with *Diaptomus*-dominated zooplankton and a substantial filamentous blue-green component in the phytoplankton.

Conclusion

Our analysis suggests that, while Lake Michigan responded to the approximately 35% P-load reduction, the response to altered zooplanktivory was much greater. These results are important to Lake Michigan water quality management as well as to the larger context of a controversy currently emerging among ecologists and limnologists. As argued by McQueen et al. (1986), the trophic state of a lake system may play a role in the relative importance of food-web interactions. This conclusion derives from analysis based on comparative studies (i.e. regression analyses of among-lake variation) and is valid in that perspective, but it does not address questions often before the researcher or manager. Those questions pertain to the potential behavior of a particular lake and/or a subset of similar lakes. At that scale, the properties of dominant species and their inter-

actions appear to become critical. In Lake Michigan, the role of large *Daphnia* is the critical component. In fact, the relative abundance of large *Daphnia* is key to most theoretical and experimental food web studies reported to date (see Carpenter et al. 1985; McQueen et al. 1986; Carpenter et al. 1987).

Although we have evaluated the importance of nutrient loading and food-web interactions as a dialectic (i.e. as competing hypotheses), that approach may be less constructive than a hierarchical one (Allen and Starr 1984; Carpenter and Kitchell 1987). Nutrient loading obviously determines the basic trophic state of a system. Within the variation of annual nutrient loads, food-web interactions in Lake Michigan regulate nutrient cycling and the composition of the plankton community. Strong effects on community composition continue to cascade from fisheries management practices and the interactions among exotic species in Lake Michigan. While anthropogenic actions may evoke equally important and unexpected effects on food webs elsewhere, it is clear that we have only begun to develop the conceptual and analytical skills required to understand, anticipate, and effectively manage the role of food web interactions. These skills will only improve through aggressive, integrated experimental and theoretical research programs.

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Factors Affecting the Time Series Bias in Stock–Recruitment Relationships and the Interaction Between Time Series and Measurement Error Bias

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Caputi, N. 1988. Factors affecting the time series bias in stock–recruitment relationships and the interaction between time series and measurement error bias. Can. J. Fish. Aquat. Sci. 45: 178–184.

The stock–recruitment relationship (SRR) and the recruitment to spawning stock relationship (RSR) of the tiger prawn (*Penaeus esculentus*) in Exmouth Gulf, Western Australia, were used to examine the time series bias and measurement error bias by Monte Carlo simulations. The bias of the SRR parameters was decreased by adding environmental variables which reduced the variation about the SRR. However, because of the many variables which may be tested and the short time series of data usually available, one needs to be careful about possible spurious correlations. The variation about the RSR also needs to be taken into account, since it has an inverse relationship with the bias in the SRR. Simulating the effect of measurement error as well as the random variation about the SRR and RSR helps to determine the possible combinations of measurement error and random variation which may result in the observed variation about the SRR and RSR. These simulations also indicate that there is an interaction between measurement error and random variation about the SRR/RSR on the bias of the parameters of the SRR. This suggests that the time series bias and measurement error should be investigated simultaneously.

Les relations stock–recrutement (RSR) et recrutement–stock de reproducteurs (RRS) chez *Penaeus esculentus* dans le golfe d'Exmouth (ouest de l'Australie) ont été utilisées pour étudier les erreurs de séries chronologies et les erreurs de mesure par simulations Monte Carlo. Les erreurs des paramètres de la RSR ont été réduites par l'addition de variables environnementales qui ont diminué la variation autour de la RSR. Cependant, à cause du grand nombre de variables qui peuvent être testées et de la durée généralement courte des séries, il faut être conscient du fait que les corrélations peuvent être fausses. Il faut aussi tenir compte de la variation autour de la RRS puisque celle-ci est en relation inverse avec l'erreur sur la RSR. La simulation des effets de l'erreur de mesure ainsi que de la variation aléatoire autour de la RSR et de la RRS a aidé à déterminer des combinaisons d'erreur de mesure et de variations aléatoires susceptibles de produire la variation observée autour de la RSR et de la RRS. Ces simulations ont aussi indiqué l'existence d'une interaction entre l'erreur de mesure et la variation aléatoire autour des valeurs de RSR et de RRS sur les erreurs des paramètres de la RSR. Ces résultats suggèrent la nécessité d'étudier simultanément l'erreur des séries chronologiques et l'erreur de mesure.

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Walters (1985) drew attention to an important source of bias present in the estimates of the parameters of a stock–recruitment relationship (SRR) fitted using the usual regression techniques. The bias arises because of the correlation between the residual from the SRR and the subsequent spawning stock. Walters used Monte Carlo simulations to show that the bias is proportional to the variation about the SRR.

In general there is considerable variation about a typical SRR with the major part of this being due to environmental factors affecting recruit survival. If these environmental factors can be identified and incorporated in the SRR, a family of SRRs (e.g. Garcia 1983) are produced, and the residual variance would be reduced. The effect on this reduction on the bias of the estimates of the parameters of the SRR is investigated by Monte Carlo simulations for the SRR of the tiger prawn (*Penaeus esculentus*) in Exmouth Gulf, Western Australia (Penn and Caputi 1985, 1986).

The effect of the variation about the replacement curve, i.e. the recruitment to spawning relationship (RSR), on the bias of the parameters of the SRR, has also been examined. Walters (1985) assumed constant fishing effort and no random variation about the RSR when examining the time series bias. Any variation about this relationship, whether due to variation in fishing effort or random variation, would weaken the relationship between the deviation from the SRR and the subsequent spawning stock and hence reduce the bias in the SRR. In fact, even the parameters of the RSR may be subject to bias because of a possible correlation between the deviations in the spawning stock levels and the subsequent recruitment.

Walters and Ludwig (1981) noted that errors in the measurement of recruitment and spawning stock can result in a bias to

TABLE 1. Percentage bias in the parameters (a, b) of the SRR and the parameters (e, f) of the RSR over a range of SDs about the SRR and RSR. The measurement error was assumed to be zero in these simulations.

SD of SRR	SD of RSR = 0				SD of RSR = 0.15				SD of RSR = 0.23				SD of RSR = 0.30			
	a	b	e	f	a	b	e	f	a	b	e	f	a	b	e	f
0							−1	−1			0	−1			−2	−2
0.20	5	8			5	7	0	0	4	6	0	1	3	5	1	1
0.38	12	17			10	15	0	0	6	11	−1	−1	9	13	0	0
0.50	12	18			12	17	1	1	10	15	0	−1	8	12	0	0

TABLE 2. Percentage bias in the parameters (a, b, c, d) of the SRR with environmental variables and in the parameters (e, f) of the RSR over a range of SDs about the SRR. The measurement error was assumed to be zero in these simulations.

SD of SRR	SD of RSR = 0.23					
	a	b	c	d	e	f
0.10	1	2	−1	0	0	0
0.17	2	4	−3	0	0	0
0.25	4	10	−6	−1	1	1

the parameters of the SRR. However, some random variability about the SRR was also considered as well as the measurement error, so that the resultant bias was due not only to measurement error as noted by Walters and Ludwig (1981) but also contained the effects of the time series bias (C. J. Walters, University of British Columbia, pers. comm.). This study systematically examines the effect of the bias arising from random variation about the SRR in combination with measurement errors to determine the interaction between these two types of bias which are common in most stock–recruitment analyses.

Ludwig and Walters (1981) presented a procedure for estimating the parameters of the SRR in the presence of measurement errors by using the ratio of environmental and measurement error variances. But this does not take into account the time series bias because in the model formulation examined, the spawning stock was not dependent on recruitment.

These simulations will also be used to examine the effect of various combinations of measurement error and environmental variation on the resultant observed variation about the SRR and RSR. These may then be used to determine possible combinations of measurement error and environmental variation about the SRR and RSR which would result in the variation actually measured about the SRR and RSR.

Stock–Recruitment Relationships

The Ricker SRR for the tiger prawns of Exmouth Gulf, fitted to 13 year-classes using a logarithmic transformation, resulted in a multiple correlation of 0.65 with a residual mean square of 0.141, i.e. standard deviation (SD) of 0.38:

$$R_t = aS_{t-1} \exp(-bS_{t-1} + W_t)$$

where R_t is the recruitment index during March–May of year t, S_{t-1} is the spawning stock index during August–October of the previous year, and W_t is a normally distributed random variable (Penn and Caputi 1986). Parameter values a and b were 4.179 and 0.07053, respectively. The effect of cyclones on tiger prawns just prior to recruitment to the fishery was then incorporated into the SRR as follows:

$$R_t = aS_{t-1} \exp(-bS_{t-1} + cJ_t + dF_t + W_t)$$

where J_t and F_t are the rainfall (millimetres) occurring in January and February, respectively, at Exmouth Gulf. Parameter values a, b, c, and d were 3.71, 0.053, −0.0029, and 0.0030, respectively. Since cyclones usually result in heavy rainfall, the rainfall has been used as an index of the intensity of the cyclone and its distance from Exmouth Gulf. This relationship resulted in a multiple correlation of 0.97 and SD about the regression of 0.17. Cyclones appear to have the potential to both increase and decrease recruit survival depending on their timing (Penn and Caputi 1985). While other factors likely to be involved in determining the influence of the cyclones are currently under investigation, the above relationship will be used as the basis for the simulations for the purpose of this study.

The replacement relationship (i.e. RSR) resulted in the following equation:

$$S_t = eR_t \exp(-fE_t + V_t)$$

where E_t is the effective effort on recruits (Penn and Caputi 1986) and V_t is a normally distributed random variable. Parameter values e and f were 1.24 and 0.0232, respectively. This was also fitted using a logarithmic transformation and resulted in a multiple correlation coefficient of 0.94 and SD about the relationship of 0.23.

Monte Carlo Simulation

Monte Carlo simulations were undertaken to detect the level of bias in the parameters of the above relationships. Each Monte Carlo analysis consisted of the simulation of 1000 data sets, each with 13 stock and recruitment values. For each data set the starting spawning stock was set to 18, the same spawning stock as was obtained at the commencement of the observed data set. The subsequent recruitment and spawning stocks were obtained by successive application of the SRR (with or without the environmental effects) and the RSR. The random variation about the relationships was assumed to be independent and lognormally distributed. The effort and environmental values for the 13 yr were set to those of the observed data (Penn and Caputi 1986).

The first simulations were similar to those of Walters (1985) except that the fishing effort varied according to the observed effort over the period rather than being constant. Since effort varied, the results should show less bias than reported for the constant-effort case by Walters. The SRR ignoring environmental effects was used with a random error from a lognormal distribution over a range of SDs (0.2–0.5) near the actual SD measured about the relationship of 0.38. The random variation

TABLE 3. Percentage bias in the parameters (a, b) of the SRR over a range of SDs on measurement error and with and without variation on the SRR and the RSR.

SD		SD on measurement error									
		0		0.1		0.2		0.3		0.4	
SRR	RSR	a	b	a	b	a	b	a	b	a	b
0	0	0	0	2	2	7	9	13	14	17	17
0	0.23	0	0	1	2	4	5	8	7	11	9
0.38	0	12	17	10	15	11	15	11	14	15	15
0.38	0.23	6	11	8	13	11	13	10	11	13	12

about the replacement curve (RSR) and the measurement error were assumed to be zero. This resulted in a bias in the density-independent coefficient a and the density-dependent coefficient b of 12 and 17%, respectively, at the SD of 0.38. The level of bias was related to the level of SD (Table 1) similar to the result of Walters.

The effect of adding random variation about the RSR was then examined using a range of SDs (0.15–0.30) near the actual lognormal SD of 0.23. This reduced the bias in the parameters of the SRR (Table 1) as expected from equation (4) in Walters (1985). The bias in the a and b parameters dropped to 6 and 11%, respectively, at the SD of 0.38 and 0.23 for the SRR and RSR, respectively. This analysis also showed that the bias in the parameters e and f of the RSR was less than 1% for the range of SDs about the SRR and RSR (Table 1).

Monte Carlo simulations were then applied to the SRR with environmental effects incorporated. This was undertaken using a range of SDs (0.10–0.25) about the actual SD of 0.17 obtained from the SRR with environmental factors and using the SD of 0.23 obtained for RSR. This showed that the level of bias in the parameters of the SRR is reduced when the unexplained variation about the SRR is reduced by the addition of environmental factors which affect recruitment to the SRR (Table 2). The bias in the a and b parameters was 2 and 4%, respectively, at the SD of 0.17 for the SRR.

The effect of measurement errors was estimated by assuming a lognormal distribution with a range of SDs (0.1–0.4) for the errors in the true recruitment and spawning stock levels. The simulations were first undertaken using the SRR with no environmental effects and using the actual SDs measured about the SRR and RSR of 0.38 and 0.23, respectively. Tnis resulted in a small increase in the bias of the a and b parameters at the lowest SD on measurement errors (0.1) from 6 and 11%, respectively, to 8 and 13%, respectively (Table 3). Further increases in the SD of measurement error resulted in further small increases in bias of parameter a. This can be contrasted with simulations allowing only measurement error and no random variation about the SRR and RSR. This shows an almost linear increase in bias over the range of SD on measurement error tested (0.1–0.4). Table 3 also illustrates the effect of measurement error with random variation about the SRR and no random variation about RSR, and vice versa. This confirms that adding the random variation about the RSR reduces the bias in the SRR even if measurement error is present.

Interestingly, at the higher levels of measurement error examined (SD of 0.3, 0.4), the bias was lower when a combination of random variation about the SRR and RSR with measurement error was tested compared with when only measurement error was allowed. This interaction between the random variation about the SRR and the level of measurement error when examining the bias in the parameters was confirmed by additional

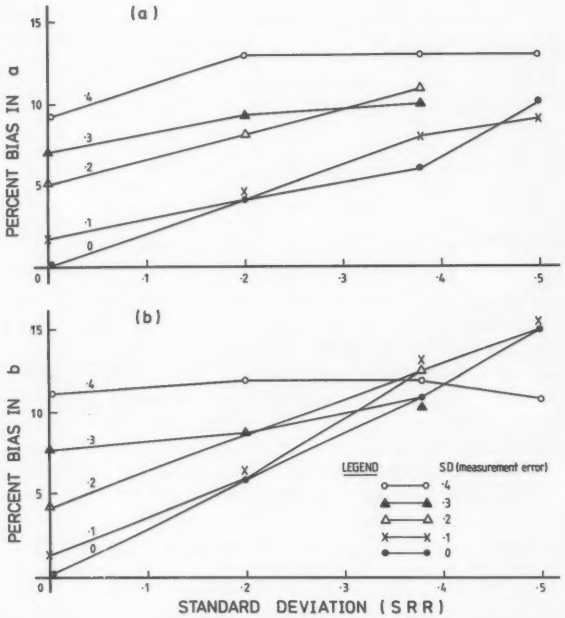

FIG. 1. Percentage bias in the parameters (a,b) of the SRR over a range of SDs on the SRR and for the measurement error (indicated on the lines). The SD for the RSR was set at 0.23.

simulations (Fig. 1). This figure shows the slope of the relationship between the bias in the parameters of the SRR when the random variation about the SRR decreases with increase in measurement error. The random variation about the RSR has been assumed to be 0.23 for all simulations in this figure.

Similar results were obtained when simulating the SRR with environmental effects and using the SD about the SRR and RSR of 0.17 and 0.23, respectively, together with measurement error. The combination of the random variation about SRR and RSR and the SD of measurement error of 0.1 resulted in a bias of 4 and 9%, respectively, for the parameters a and b (Table 4). This compares with bias of 2 and 4% for the simulations allowing random variation about the SRR and RSR only.

These simulations can also be used to determine the resultant residual random variation arising from the various combinations of measurement error and random variation (mainly due to the environment) about the SRR and RSR. Figure 2a shows the mean residual SD of the SRR for input SD of the SRR in the simulations and the assumed SD on the measurement error with the SD on the RSR set at 0.23.

TABLE 4. Percentage bias in the parameters (a, b) of the SRR with environmental variables over a range of SDs on measurement error and with and without variation on the SRR and RSR.

SD		SD on measurement error									
		0		0.1		0.2		0.3		0.4	
SRR	RSR	a	b	a	b	a	b	a	b	a	b
0	0	0	0	2	4	8	17	14	26	24	41
0.17	0.23	2	4	4	9	9	18	13	25	18	33

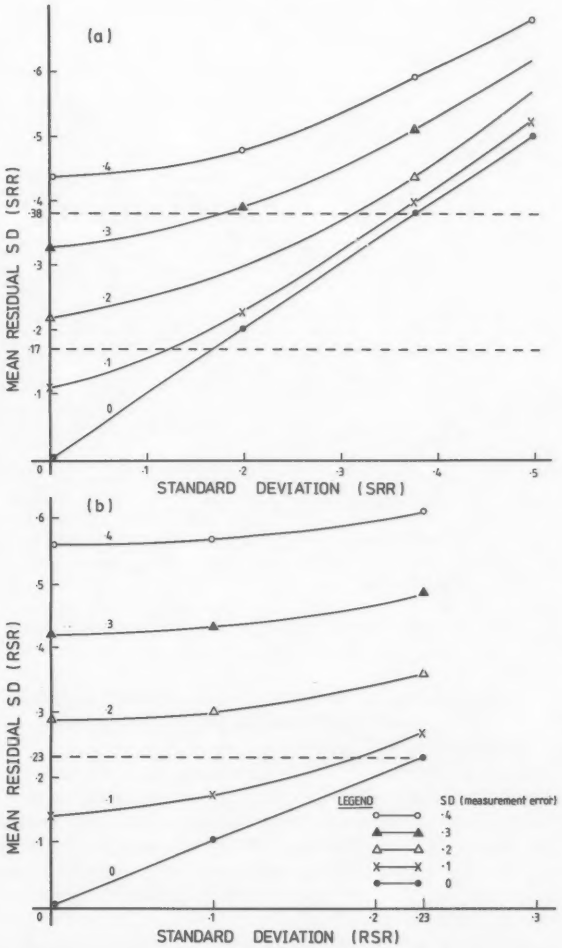

FIG. 2. (a) Mean residual SD of the SRR from the Monte Carlo simulations for a given input SD on the SRR (x-axis) and a given SD on the measurement error (adjacent to the curve). The SD on the RSR was set at 0.23 for all simulations. The broken horizontal lines show some possible combinations of measurement error and variation about the SRR resulting in the observed SDs of 0.38 and 0.17 about the actual SRR. (b) Variation in the mean residual SD of the RSR for a given input SD on the RSR (x-axis) with the SD on the SRR set at 0.38 for all simulations. The broken horizontal line shows the observed SD of 0.23 about the actual RSR.

The position of the observed SD about the SRR represented by the broken horizontal line at 0.38 indicates that a range of possible combinations of measurement error and environmental variation about the SRR exist which would result in the observed SD. That is, for this SRR it is possible that the observed variation about the SRR is due to a small amount of measurement error (e.g. SD of 0.1) and a large component of environmental variation (SD of 0.36) or due to a high level of measurement error (e.g. SD of 0.3) and a corresponding lower environmental variation (SD of 0.18). Only measurement errors associated with a SD of 0.4 appear to be an overestimate. (There is no combination of this measurement error with environmental variation which results in the observed SD of 0.38).

TABLE 5. Percentage bias in the parameters (a, b) of the SRR for four combinations of input SDs for measurement error, SRR, and the RSR which are expected to result in a SD of 0.38 and 0.23 for simulated SRR and RSR, respectively.

Input SD			Simulation results			
			SD		Percent bias	
Measurement error	SRR	RSR	SRR	RSR	a	b
0	0.38	0.23	0.38	0.23	6.2	10.5
0.05	0.37	0.21	0.38	0.22	8.7	12.5
0.10	0.36	0.18	0.37	0.23	9.1	13.0
0.15	0.34	0.05	0.38	0.22	10.2	13.7

However, once an environmental variable that affects recruitment is identified and incorporated in the SRR, then the SD about the relationship, which includes a combination of measurement error and environmental variation, is reduced in this case to 0.17. This now implies that the SD of the measurement error is about 0.1, while the remaining environmental variation about the SRR has a SD of less than 0.17.

Similarly, Fig. 2b shows the mean residual SD of the RSR from the simulations with a range of SDs about the RSR and a range of possible measurement errors, with the SD about the SRR set at 0.38. This figure confirms that the SD on measurement error is unlikely to be 0.2 or greater, since the resultant variation about the RSR would be at least 0.29, i.e. higher than the SD estimated from the actual data set of 0.23. Figure 2 can thus be used to determine some combinations of the SD on measurement error and random variation (mainly due to environmental effects) on the SRR and RSR that are compatible with the observed variation about these relationships (see Tables 4 and 5).

Table 5 shows a number of possible combinations of measurement error and environmental variation which should result in a mean SD about the simulated SRR and RSR of 0.38 and 0.23, respectively. The actual simulations confirm this assumption and also show the bias in the SRR. Table 6 shows a similar result for the SRR with environmental parameters incorporated.

Bias Correction

The results of the Monte Carlo simulations can also be used to estimate the parameters of the "true" SRR by adjusting the

TABLE 6. Percentage bias in the parameters (a, b) of the SRR with environmental variables for four combinations of input SDs for measurement error, SRR, and the RSR which are expected to result in a SD of 0.17 and 0.23 for the simulated SRR and RSR, respectively.

Input SD			Simulation results			
			SD		Percent bias	
Measurement error	SRR	RSR	SRR	RSR	a	b
0	0.17	0.23	0.17	0.23	1.6	4.2
0.05	0.15	0.21	0.16	0.22	1.9	5.1
0.10	0.13	0.18	0.17	0.23	2.4	5.5
0.15	0.03	0.05	0.17	0.22	5.1	10.0

parameters of the fitted SRR (Walters 1985), although Walters and Staley (1987) indicated that the Monte Carlo procedure does not always provide a proper bias correction. Thus, assuming no measurement error, the parameters a and b of the SRR with no environmental variables can be reduced by 6.2 and 10.5% (Table 5) to 3.94 and 0.064, respectively. Similarly, for the SRR with environmental variables incorporated, the a and b parameters may be adjusted by 1.6 and 4.2% (Table 6), respectively, to 3.65 and 0.051. Thus, even with this correction for bias there is a 20% decrease in the density-dependent parameter for the SRR with environmental variable compared with that without.

To examine one aspect of measurement error, the variation about the mean catch rates for recruitment and spawning stock in the 1980 log book data were examined. While there is more variation between individual catch rates during the recruitment phase of the fishery compared with the spawning phase, there are generally more boat days of fishing during the recruitment phase (and hence more samples) resulting in a standard error for recruitment and spawning index of less than 2% of the estimate. This corresponds to a SD of measurement error of about 0.02. However, the major source of measurement error is probably due to the accuracy of the catch rate data in estimating the true index of abundance of recruitment and spawning stock. For the purpose of this study a SD of measurement error of 0.1 will be assumed to reflect the overall measurement error. Thus, for the SRR with no environmental variables the a and b parameters would be reduced by 9.1 and 13.0% (Table 5) to 3.83 and 0.062, respectively, while for SRR with environmental variables the parameters would be reduced by 2.4 and 5.5% (Table 6) to 3.62 and 0.050, respectively.

Discussion

In the Monte Carlo simulations, the initial spawning stock and the fishing effort have been maintained at the values obtained in the fishery. This enables the effect of the random variation and measurement error on bias in the SRR for this tiger prawn fishery in Western Australia to be evaluated. When assessing the bias in the SRR for a particular fishery, it is important to include the starting stock size and the pattern of fishing over the period of the stock–recruitment analysis. Changing the fishing effort, for example, would generate stock and recruitment values which are not comparable with the original data values. Hence, there would be differences between the actual and generated SRR, not only due to the random variation about the SRR but also due to the changes in the fishing pattern. This approach still enables some trends in the bias of SRRs to be assessed concerning the effect of the environmental variables

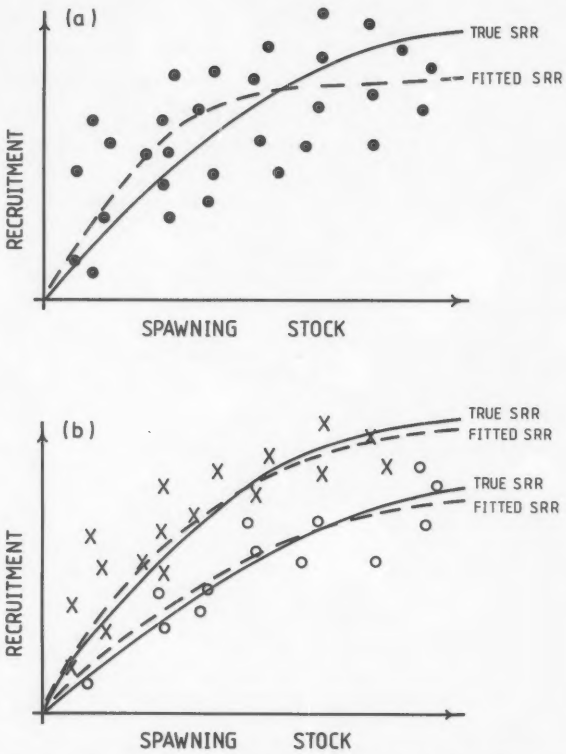

FIG. 3. (a) Typical scatter of points in a SRR with the fitted and "true" relationships shown. (b) Same scatter points with an environmental factor identified (× and ○ representing favourable and unfavourable environmental conditions, respectively) and a multiple regression relationship used to fit the effect of the environmental factor and spawning stock on recruitment.

and variation about the RSR. These trends would be generally applicable to stock–recruitment analysis, although the actual level of bias would differ for each particular case.

The application of the Monte Carlo does not always provide a proper correction of bias; for example, the bias in the b parameter of the SRR may not be properly assessed when the spawning stock values are generally low (Walters and Staley 1987). However, the spread of the spawning stock values in the Exmouth Gulf tiger prawn fishery indicates that this should not be a problem in this case.

The time series bias in the parameter estimates arises because of the correlation between W_t and the subsequent spawning stocks S_t, S_{t+1}, etc., and is related to the level of variation about the relationship (Walters 1985). The bias can thus be reduced by the correct identification of environmental variables which explain some of the recruitment variation (i.e. reduce the variation in W_t). Thus, even though the variation about a SRR may be high, if the source of the variation can be identified and incorporated into the relationship (see Fig. 3), then not only will the SRR fit better but the bias in the estimated parameters of the SRR will be reduced.

However, care should be exercised in the addition of environmental variables to a SRR, as the significance of the environmental variable may be spurious. This could occur quite often in view of the many environmental variables which may

be tested and the short time series associated with stock–recruitment analyses. In this situation the addition of environmental variables will result in an artificial reduction in the variance about the SRR and hence an apparent low bias when in fact only more variance has been added to the parameters of the SRR. Thus, before adding an environmental variable to the SRR, the consequences of the correlation being real or spurious need to be carefully evaluated.

While the time series bias is present in all SRRs which are based on natural variation in spawning stock, it would be particularly strong in a species with a 1-yr life cycle and subject to large environmental variation such as some prawn species. This could partly be the reason why previous studies have been unable to show SRR for prawns (Garcia 1983) and highlights the importance of correctly assessing both the environmental variable and the spawning stock in SRRs.

When assessing the bias in the parameter estimates, it is important to consider the effect of fishing effort (E_t) and the variation in the RSR (i.e. V_t). This variation is inversely related to the bias in the parameters of the SRR (Table 1). The variation in the RSR is generally smaller than for the SRR because the effect of the environment on the egg and larval phase of the population is much greater than during the juvenile and adult phase. Probably the major source of variation from the recruitment to spawning stock for most fisheries is the effect of the fishing effort. Hence, it is the combined effect of E_t and V_t which reduces the time series bias. This reduction occurs because the relationship between the deviations from the SRR (i.e. W_t) and the subsequent levels of the spawning stock (S_t, S_{t+1}, etc.) is weakened and it is this relationship which is the cause of the time series bias (equation (4) in Walters 1985).

The bias in estimates of the parameters e and f of the RSR was minimal and so could generally be ignored (Tables 1 and 2). The lack of bias in the parameters of this relationship is at first sight surprising, since the range of SDs considered about this relationship is similar to the range of SDs examined about the SRR with the environmental variables. This latter analysis produced a bias of approximately 6%. However, any correlation between the deviations from the RSR (V_t) and the subsequent recruitment (R_{t+1}, R_{t+2}, etc.) would be greatly reduced by the variation of the recruitment in the SRR caused by environmental effects and the random variation (W_t), and so would minimise the bias.

The addition of some measurement error to the estimates of recruitment and spawning stock abundance resulted in a small increase in the bias of the parameters of the SRR. When the effect of measurement error was examined in the absence of random variation about the SRR and RSR, there was a sharper increase in bias with increase in measurement error (Table 3). Thus, there is an interaction between measurement error and random variation about the SRR/RSR on its effect on the bias of the parameters of the SRR. This interaction was also illustrated in Fig. 1 which indicates that the level of random variation has a stronger influence on the bias at low levels of measurement error. At high levels of measurement error there is only a small increase in bias with increase in variation about the SRR. Hence, it is important to evaluate the effect on the bias of the random variation about the SRR and measurement errors simultaneously.

In general the variation measured about the fitted SRR and RSR is unlikely to be due solely to environmental factors; part of this variation will be due to measurement error. Thus the "true" random variation about SRR and RSR may be less than that measured from the fitting of the SRR and RSR. In practice the combination of large measurement error and small random variation would not occur, since a large measurement error of the stock and recruitment levels would result in relatively large "random" variation about the fitted SRR and RSR (Fig. 2). Hence the range of viable combinations of SD on the measurement error and environmental variation about the SRR and RSR is greatly reduced. A number of these combinations may then be examined to determine the possible level of bias in the fitted SRR so that the parameters may be corrected closer to the "true" SRR. If estimates of measurement error were obtained, as suggested by Ludwig and Walters (1981), this would further pinpoint the range of likely combinations.

The measurement errors for both recruitment and spawning stock have been assumed to be the same in this study, but this may not generally be the case. In this situation the effect of the different combinations of measurement error on recruitment and spawning stock would have to be assessed.

The actual level of bias noted in this study was not excessively high, especially when the effect of environment was incorporated into the SRR. However, this undoubtedly is due to the good fits obtained in the SRR and RSR, which are probably better than is usually obtained in stock and recruitment analysis. Most SRRs are characterised by a wide scatter of points, due to a combination of environmental effects on recruit survival and measurement error, and so would be prone to larger biases in the SRR than noted here.

The importance of examining the bias in the SRR was stressed by Walters and Ludwig (1981) and Walters (1985). They noted that the effect of both of the biases was to make the recruitments appear to be independent of spawning stock, a conclusion which could be disastrous for the proper management of the fisheries. For example, an upward bias in the a and b parameters of the SRR, as obtained in all simulations presented in this paper, would result in an overestimate of the optimum exploitation rate and an underestimate of the optimum spawning stock when attempting to maximise catch (Walters 1985). However, the effects of the measurement error and time series bias are not additive, so that to assess the overall bias in the stock–recruitment analysis, these biases should be examined simultaneously.

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NOTES

Effects of Initial Conditions on Monte Carlo Estimates of Bias in Estimating Functional Relationships

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Kope, R. G. 1988. Effects of initial conditions on Monte Carlo estimates of bias in estimating functional relationships. Can. J. Fish. Aquat. Sci. 45: 185–187.

Some of the results presented by Walters (1985. Can. J. Fish. Aquat. Sci. 42: 147–149) for the magnitude of bias in estimating functional relationships from time series data resulted from his choice of initial stock size in Monte Carlo simulations rather than the dynamics of the model. Walters used the same initial stock size in each simulation while varying parameters in the stock–recruitment relationship. Starting each simulation at the equilibrium stock size or allowing initial stock size to vary randomly produces larger estimates of bias and leads to different conclusions about the relationship of bias to parameter values in the model.

Certains des résultats présentés par Walters (1985. Can. J. Fish. Aquat. Sci. 42 : 147–149) sur l'importance de l'erreur dans l'estimation des relations fonctionnelles à partir des données de séries chronologiques sont dues à la taille initiale du stock choisie par l'auteur pour les simulations Monte Carlo et non à la dynamique du modèle. Walters a utilisé la même taille initiale du stock pour toutes les simulations, tandis qu'il a fait varier les paramètres de la relation stock–recrutement. Lorsqu'on commence toutes les simulations avec la taille d'équilibre du stock ou qu'on laisse la taille initiale du stock varier aléatoirement, les erreurs estimées sont plus importantes et on aboutit à différentes conclusions concernant la relation entre l'erreur et les valeurs des paramètres dans le modèle.

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Walters (1985) demonstrated the bias in estimating functional relationships from short time series of data using the Ricker stock–recruitment relationship as an example. He correctly identified the cause of this bias, but in his discussion of the magnitude of the bias, some of his results were misleading because they depended on the initial conditions of his Monte Carlo simulations rather than on the dynamics of the model he used.

The model used by Walters consists of two equations:

$$(1)\quad R_t = S_{t-1} \exp(a - bS_{t-1} + W_t)$$

$$(2)\quad S_t = s_t R_t$$

where R_t is recruitment at time t, S_t is spawning stock at time t, a and b are parameters that determine the shape of the stock–recruitment curve, s_t is the survival rate from recruit to spawner (1 − harvest rate), and W_t are normally distributed random variables with mean 0.0 and standard deviation σ_w. Walters used a constant harvest rate of 0.5 so that $s_t = s = 0.5$. These equations comprise a model in which recruitment and spawning escapement are assumed to be measured without error, the population is completely semelparous, survival from recruits to spawners is constant, and there is random variability in recruitment that is lognormally distributed and multiplicative.

Walters used Monte Carlo simulations to estimate the bias that results from using linear regression to estimate the parameters a and b from short time series of data. To do this he generated 1000 artificial data sets, each consisting of 10 stock–recruitment pairs. He then used standard linear regression of $\ln(R_t/S_{t-1})$ on S_{t-1} to estimate a and b from each data set and used the means of the 1000 trials to estimate the bias in the parameter estimates. To construct the data sets, Walters used $a = 1.0$, specific values of b ranging from 0.3 to 1.0, specific values of σ_w ranging from 0.0 to 1.0, and S_0 (the initial value of spawners) $= 1.0$.

Walters reported that the bias in estimating a and b was nearly independent of the value of b and increased strongly for values of σ_w greater than 0.1. He stated that a was typically overestimated by about 30% and b was overestimated by a similar

TABLE 1. Relationship between σ_w and bias in parameter estimates with S_0 set equal to the equilibrium value in each trial. Parameter estimates are the average of 1000 trials of 10 data pairs each, with $a = 1.0$, $s = 0.5$, and $b = 1.0$. Standard deviations of the distributions of the parameter estimates are listed as s_a and s_b.

σ_w	Avg. $\hat{a}$	Avg. $\hat{b}$	s_a	s_b
0.001	1.33	2.06	0.33	1.07
0.100	1.34	2.09	0.32	1.07
0.250	1.32	2.06	0.32	1.09
0.500	1.31	2.09	0.36	1.24
0.750	1.29	2.22	0.38	1.52
1.000	1.25	2.18	0.44	1.84

TABLE 2. Relationship between σ_w and bias when S_0 varies randomly. All parameter values were the same as in Table 1, but S_0 in each trial was determined by equation (2) from the final recruitment in the previous trial. S_0 in the first trial was obtained by starting at the equilibrium value and calculating 100 subsequent stock levels to remove the effect of the fixed initial value.

σ_w	Avg. $\hat{a}$	Avg. $\hat{b}$	s_a	s_b
0.001	1.29	1.94	0.32	1.04
0.100	1.31	2.01	0.31	1.05
0.250	1.28	1.99	0.30	1.14
0.500	1.30	2.36	0.35	2.10
0.750	1.29	3.15	0.37	14.86
1.000	1.28	28.96	0.43	239.43

amount for values of σ_w in the range 0.5–1.0. These biases resulted in overestimating the optimal harvest rate by 30% and underestimating the optimal spawning escapement by 50%. However, these results are not a general characteristic of the model and fitting technique, but instead result from the systematic choice of S_0.

An understanding of how the choice of S_0 affects the estimated bias can be gained by combinig equations (1) and (2) to describe the process as an autoregressive model:

$$(3) \quad S_t = sS_{t-1} \exp(a - bS_{t-1} + W_t)$$

or in an autoregressive form that is equivalent to the transformation used to estimate the Ricker stock–recruitment relationship by linear regression:

$$(4) \quad \ln(S_t/S_{t-1}) = a + \ln(s) - bS_{t-1} + W_t.$$

Since survival from recruitment to spawning escapement is constant, using this form for the regression estimates of a and b will not alter the results of the regression.

From equation (4) it is clear that the equilibrium value of escapement is $S_{eq} = \{a + \ln(s)\}/b$. Changing the value of b is equivalent to changing the value of S_0 relative to S_{eq}. *With* $a = 1.0$ and $s = 0.5$, the equilibrium value occurs at $S_{eq} \approx 0.307/b$. If $b = 0.3$, then $S_0 = 1.0$ is very close to the equilibrium value, but when $b = 1.0$, it is more than three times the equilibrium value. This means that for small values of σ_w, the first point in the data series contains much more information about the shape of the stock–recruitment curve than subsequent values. Consequently, the bias is significantly underestimated. If S_0 is set equal to S_{eq} in each trial, then the bias is completely independent of b and nearly independent of the choice of σ_w (Table 1).

However, this is not the most natural way of choosing initial values. The purpose of the simulations is to investigate the estimation problem that fishery researchers encounter with real data sets. Presumably, the initial value in a stock–recruitment data set is the result of the same dynamics and variability that have governed the population while data were collected. Consequently, a more realistic approach is to let the dynamics of the population determine the initial value by using equation (2) to calculate S_0 in each trial from the terminal recruitment value in the previous trial. This approach effectively generates one very long data series, and then estimates the stock–recruitment parameters from sequential segments of the longer series. Allowing S_0 to vary in this manner gives results that are very different from those obtained using a constant S_0 (Table 2). With values of σ_w greater than 0.5 the bias in $\hat{b}$ increases dramatically as does the variability in $\hat{b}$. This happens because of the multiplicative, lognormal error term in equation (1). Occasionally the variability in recruitment causes the population to become trapped at very low values (e.g. $S_t < 0.001$) for all data points in a trial. When the population is trapped at such a low value, the variability in $\ln(R_t/S_{t-1})$ remains about the same, but the variability in S_t is compressed to a very small range near the origin. This results in occasional regression estimates of b that are extremely large. Consequently, results from replicate Monte Carlo simulations with large values of σ_w may differ substantially from the examples listed in Table 2. Fixing the value of S_0 obscures this behavior of the model because the population is reset to the same initial value in each trial.

Another point of interest that is apparent from equation (4) is that the constant estimated by linear reqression is $a + \ln(s)$ rather than simply a. Changing the survival rate in this model has the same effect on bias as changing the value of a. If a and s are both changed so that the quantity $a + \ln(s)$ is constant, then the absolute bias in $\hat{a}$ and $\hat{b}$ and the variability of these estimates remains unchanged. For example, using $a = 0.31$ and $s = 1.0$ with $\sigma_w = 0.5$ would produce the same bias (i.e. overestimation of a by 0.31) as using $a = 1.0$ and $s = 0.5$ (Table 2).

Although the bias in estimates of stock–recruitment relationships is of interest, it is probably overshadowed in practical applications by the variability in these estimates. Because the bias results from the correlation between deviations from expected recruitment and subsequent spawning stocks, variability in survival from recruitment to maturity should reduce the bias, but may have mixed effects on the variability of parameter estimates. If survival varies in a purely random manner, the correlation between recruitment and subsequent stock levels will decrease and variability in the spawning stock will increase. Consequently, both bias and variability in parameter estimates should decrease, but because random survival will have a greater relative effect on the correlation between recruitment and subsequent stock than on stock variability, it is unlikely that the decrease in variability will be as great as the decrease in bias (cf. Caputi 1988). If survival is compensatory (either from density-dependent mortality or as the result of management for fixed escapement goals), then bias should still decrease, but because the variability in spawning stock is reduced relative to the variability in recruitment, the variability in parameter estimates may increase.

Much of our intuitive knowledge about estimation problems is based on linear models, but when dealing with nonlinear models, intuition may be a poor guide. Since stock–recruitment relationships are routinely estimated from data series of limited length, and are frequently used in formulating management policies, the estimation problem deserves to be investigated in

depth. This problem should be approached analytically as far as possible, and if Monte Carlo simulations are used, great care should be exercised in examining the assumptions of the model and the initial conditions.

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A Reevaluation of the Search Cycle of Planktivorous Arctic Grayling, *Thymallus arcticus*

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Evans, B. I., and W. J. O'Brien. 1988. A reevaluation of the search cycle of planktivorous arctic grayling, *Thymallus arcticus*. Can. J. Fish. Aquat. Sci. 45: 187–192.

Planktivorous arctic grayling, *Thymallus arcticus*, were previously believed to forage using a cruise strategy, searching for prey while swimming, while other planktivores have been found to search while stationary, a strategy termed "saltatory search." Comparing the predicted components of cruise and saltatory search, we observed that the planktivorous feeding behavior of the grayling was more consistent with the strategy of saltatory search. Although the grayling appear to swim continuously, they actually pause before making turns. Swim speed is reduced with decreased prey detectability, as would be expected of a cruise searcher; however, this behavior is believed to be a result of fin placement and body form constraints. Pause duration, run length, and run turn angle were observed to vary when grayling fed on prey of different sizes, all in a manner consistent only with saltatory search. The relationship between the ability to locate prey and the components of search suggests that the grayling alter the way they search for different prey, possibly as a means of increasing search efficiency.

Il était reconnu que l'ombre arctique (*Thymallus arcticus*) planctivore recherchait ses proies en fouillant tout en nageant tandis que d'autres espèces planctivores les recherchent en demeurant sur place; cette dernière stratégie d'alimentation a été nommée recherche saltatoire. En comparant les composantes extrapolées des recherches déambulatoire et saltatoire, les auteurs ont observé que le comportement alimentaire planctivore de l'ombre arctique concordait mieux avec la stratégie de recherche saltatoire. Quoique l'ombre arctique semble nager continuellement, il s'arrête un instant avant de changer de direction. Il réduit sa vitesse de nage en fonction d'une baisse du niveau de détection d'une proie, comme l'on doit s'attendre chez un chercheur déambulatoire; toutefois, on croit que ce comportement est le résultat de l'emplacement des nageoires et de restrictions entraînées par la forme du corps. La durée de la pause, la longueur du parcours et l'angle de virage en fin de parcours varient quand l'ombre arctique recherche des proies de différentes tailles; cette variation ne concorde qu'à une recherche saltatoire. La relation entre la capacité de détection d'une proie et les composantes de la recherche porte à croire que l'ombre arctique modifie son comportement selon la proie recherchée; il est possible que ceci constitue un moyen d'accroître l'efficacité de la recherche.

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The arctic grayling, *Thymallus arcticus*, is an important gamefish common in the North American arctic and subarctic, often growing to 40 cm total length (TL) when mature. Throughout its range it is found in both lakes and rivers, spawns in small streams when the ice breaks up, and returns to the rivers and lakes after spawning (Scott and Crossman 1973). River-dwelling grayling feed on a variety of macro-invertebrates, while lake-dwelling grayling feed on zooplankton until they reach approximately 15 cm (Schmidt and O'Brien 1982), at which point they become benthic feeders (O'Brien et al. 1979). If large-bodied zooplankton are avail-

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able in lakes, large grayling will continue to feed on large zooplankton (B. I. Evans and W. J. O'Brien, unpubl.).

Previous analysis of the planktivorous feeding behavior of the grayling (Schmidt and O'Brien 1982: Evans 1986) suggested that they were typical cruise searchers, swimming continuously through the water in search of prey. In fact, it has commonly been assumed that cruise search is the strategy employed by many visually oriented planktivorous fish (Ivlev 1961; Rosenthal and Hempel 1970; Confer and Blades 1975; Janssen 1982). Recent work, however, has demonstrated that white crappie, *Pomoxis annularis* (Centrarchidae), actually utilise a stop and go search strategy termed "saltatory search" (O'Brien et al. 1986). Bluegill sunfish, *Lepomis macrochirus*, have also been observed to forage in a stop and go fashion (Janssen 1982; Evans 1986). Grayling, however, seem unlikely candidates for using saltatory search because they do not make obvious stops. A detailed analysis, however, revealed that planktivorous grayling employed a feeding behavior similar to that of white crappie.

The feeding behavior of a forager utilising a cruise search strategy would differ in several important ways from that of a forager utilising saltatory search. Cruise searchers would not pause while searching for prey, but search continuously as the animal moves; if they do pause to orient towards a located prey, then all pauses should be followed by pursuit. Saltatory searchers pause to search, and thus pause frequently; however, because not all searches are successful, not all pauses will be followed by pursuit. As a consequence of their motion, the visual discrimination ability of cruise searchers may be impaired (Gendron and Staddon 1983); therefore, they should decrease their search speed when foraging on small or cryptic prey (Gendron 1982). Saltatory searchers, in contrast, can swim at speeds independent of visual constraints because they search while stationary (O'Brien et al. 1986; Evans 1986). A cruise searcher moves continuously through the environment and should encounter prey at the periphery of the visual field, or at the maximum location distance, commonly referred to as the "reaction distance." In contrast, a saltatory searcher, by the nature of its run and stop behavior, would detect prey throughout the visual field. Cruise searchers might detect a prey soon after a run was initiated; thus, some of the runs prior to pursuit would be short. Runs by saltatory searchers would not be foreshortened prior to pursuit; furthermore, their runs function to reposition the forager to search new water, so run lengths should be shorter when they feed on small or cryptic prey than those for conspicuous prey. Cruise searching foragers would be expected to swim straight ahead and turn infrequently. However, saltatory foragers turn after a search pause to enter new, unsearched water. Furthermore, such turns should increase when large prey are present, if a larger angle is searched.

In the following analysis, we show that the components of the feeding behavior of arctic grayling are consistent with those predicted for saltatory search.

Methods

Research was conducted at or near the Toolik Lake Research Station located on the shores of Toolik Lake, Alaska (68°N, 143°W). Arctic grayling were collected either by shoreline seining from Toolik Lake or from the outflow of Lake E-1, by herding the fish into nets.

The feeding behavior of the grayling was observed in a 122-cm square tank, filled to a depth of 30 cm. The fish were filmed using a JVC-100 color television camera mounted 1.5 m above the tank. The tank was illuminated, with natural light, above 300 lx, and all experiments were performed during the early afternoon. A total of four juvenile fish (age 2), ranging from 13 to 15 cm total length (TL), were observed at temperatures of 5, 10, and 15°C and were not fed for 24 h prior to observations.

Zooplankton were collected and added to the tank at the same ambient density and species composition as found in Toolik Lake, or E-1, a lake near Toolik Lake (Evans 1986). The Toolik zooplankton community was dominated by the large-bodied (3–4 mm) calanoid copepod *Heterocope septentrionalis* and the smaller bodied (0.5–1.5 mm) calanoid *Diaptomus pribilofensis*. *Cyclops scutifer*, a cyclopoid copepod, and lower densities of other species were also present. The E-1 zooplankton community was composed of the small-bodied (0.5–1.5 mm) cladocerans *Daphnia longiremis* and *Bosmina longirostris* as well as *Diaptomus pribilofensis*. These populations are typical for these lakes (O'Brien et al. 1979).

While the fish were feeding, one or two observers described their activities onto the audio portion of the videotape. Two fish were used in each experiment, unless agressive interactions were observed. Occasionally, the fish attacked prey on the surface or bottom of the tank; these cases were noted and omitted from the analysis. Once filmed, the feeding behavior was analysed from the videotapes using a Panasonic 6300 VCR. Frame-by-frame analysis was accomplished at the University of Kansas with a Panasonic NV-A505 remote control to the VCR.

The specifics of the components of the search cycle were determined from the videotapes (O'Brien et al. 1986; Evans and O'Brien 1986; Evans 1986). Pursuit angles and distances, run lengths, and run turn angles were marked on the television screen and measured. The pursuit angle was defined as the number of degrees away from the forward direction of the fish (defined as 0° throughout) where a prey was located. Run turn angles refer to the angular change in body orientation between runs when no prey were located. The duration of the various behaviors were timed, either with a Cronus digital stop watch (0.1-s resolution) or by counting frames (0.03-s resolution).

Run lengths were defined as the distance travelled between turns. Distances were converted to centimetres by using the ratio of the actual fish length to the television fish length. Run speeds were then calculated using the run length and the time between turns. At the end of each run, and just prior to a turn and subsequent run, grayling hesitate briefly. The duration of this "pause" was measured using frame-by-frame analysis from the time the fish made no further forward progress in a run until just before the fish began to turn its head to begin the next run.

The longitudinal body axis of the fish was used as the central axis of the forward-directed visual field. When a prey was encountered, the pursuit angle was estimated as the angle between the central axis of the fish, prior to pursuit, and the line connecting the fish's nose to the position of the prey. Pursuit angles and distances were plotted on polar coordinate graph paper to estimate the horizontal component of the search volume. Right and left turns were combined and expressed as a scattergram and also as a frequency distribution of the angle of attacks in 10° increments.

A minimum of 20 data points were measured at each temperature to give a mean swimming speed and were plotted with the 95% C.I. Linear regressions were compared using *t*-

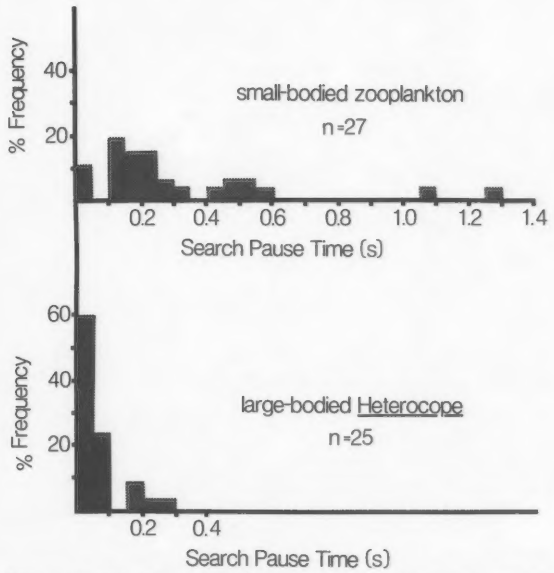


FIG. 1. Search pauses. The duration of the search pauses are illustrated for small prey (top panel) and large prey (bottom panel). Measurements were taken at 10°C.

test and GT-2 test statistics. Distributions of all other data were compared using the Kolmogorov–Smirnov two-sample test (K–S test, Sokal and Rohlf 1981).

Results

The following behavioral observations are generally consistent with the idea that arctic grayling search for zooplankton using a saltatory search strategy.

Grayling, when casually observed, appeared to swim continuously while feeding; however, slow-motion video (frame by frame) revealed a brief hesitation at the end of each run, immediately before the turn and initiation of the next run. These pauses were longer in the presence of small prey than in the presence of large prey. The mean duration of this pause was 0.07 s, with 80% of the pauses being less than 0.1 s, when feeding on large prey, and 0.3 s, with 90% of the pauses being greater than 0.1 s, when feeding on small prey (Fig. 1). These two distributions differed significantly (K–S test, $p < 0.001$). The observation that the grayling did not swim continuously suggested that, like the white crappie, they were saltatory searchers.

When feeding on large prey, the grayling swam considerably faster than they did when feeding on small prey (GT-2 test, $p < 0.05$; Fig. 2). It has been suggested that continuously searching foragers should adjust their search rate relative to the detectability of their prey (Gendron 1982; Gendron and Staddon 1983). Thus, our observations are more consistent with the idea that grayling are cruise searchers. However, if the grayling were searching during the runs, one would also predict that while the run speed should be limited by prey detectability, the pursuit speed should not (O'Brien et al. 1986). The grayling, however, showed no significant difference between run and pursuit speeds when feeding on large or small prey (GT-2 test, $p > 0.01$; Fig. 2), an observation consistent with saltatory search.

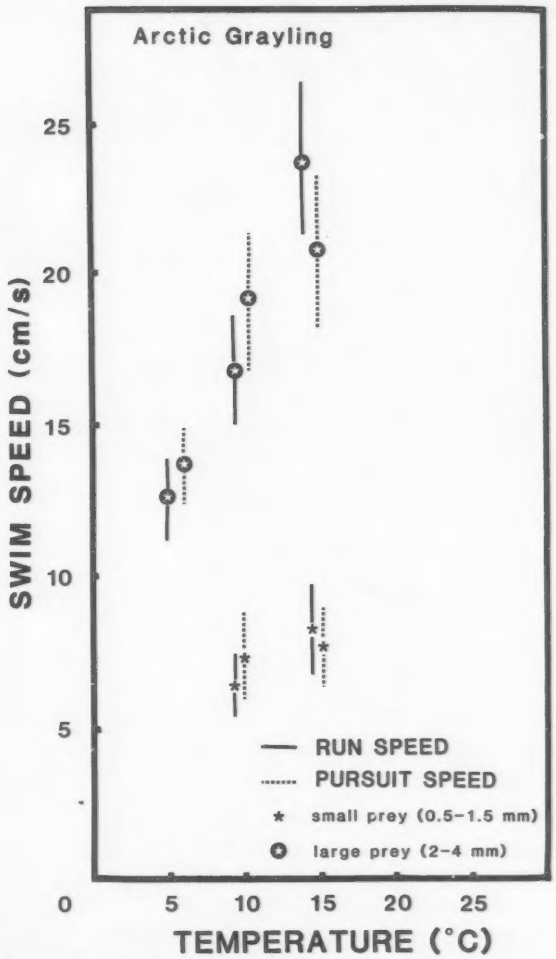


FIG. 2. Swim speed. Swimming speeds are shown for large prey at three temperatures and small prey at two temperatures. Points are mean values and bars indicate 95% C.I.

The observed search volumes of the grayling (Fig. 3c and 3d) were also inconsistent with the predictions for a cruise searching forager. Instead of pursuits being at the periphery of the search volume, as one would expect of cruise search, the pursuit lengths were distributed throughout the search volume: from 4 to 30 cm for large prey and from 4 to 15 cm for small prey. The angle at which prey were located in the search volume also varied with changes in prey size (Fig. 3a and 3b). When feeding on large prey, the majority of pursuits were within 90° of forward directed, with some pursuit angles as large as 150°. While feeding on small prey, the pursuit angles were concentrated to within 50° of forward, with a few observations extending to 70°. These two distributions differed significantly (K–S test, $p < 0.001$).

The frequency distribution of the runs lengths which were followed by another run did not differ significantly from the distribution of run lengths which were followed by pursuits, for either large or small prey (K–S test, $p > 0.05$; Fig. 4). Thus, the runs were not foreshortened prior to pursuit, which would be expected if the grayling were locating prey while

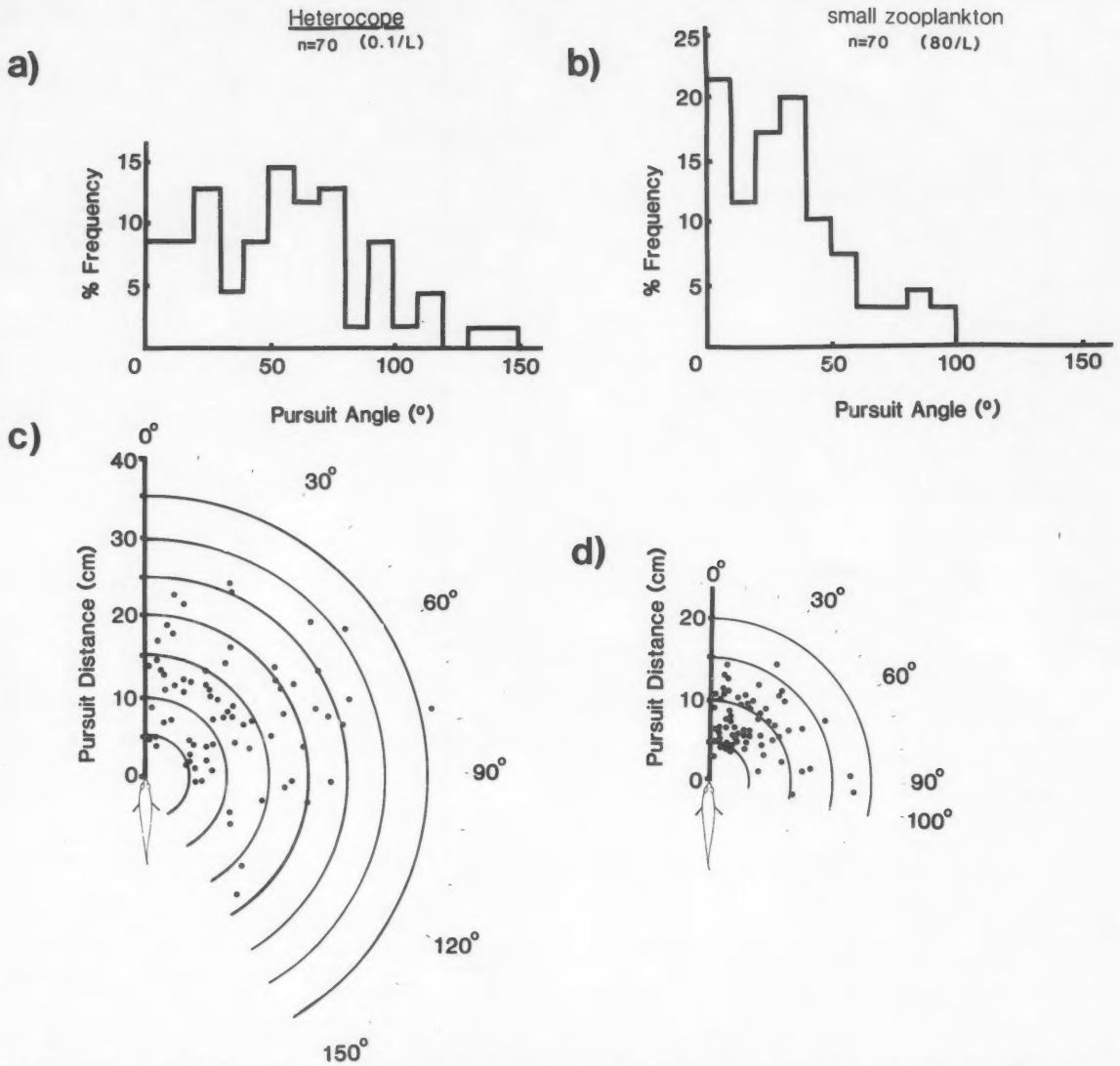


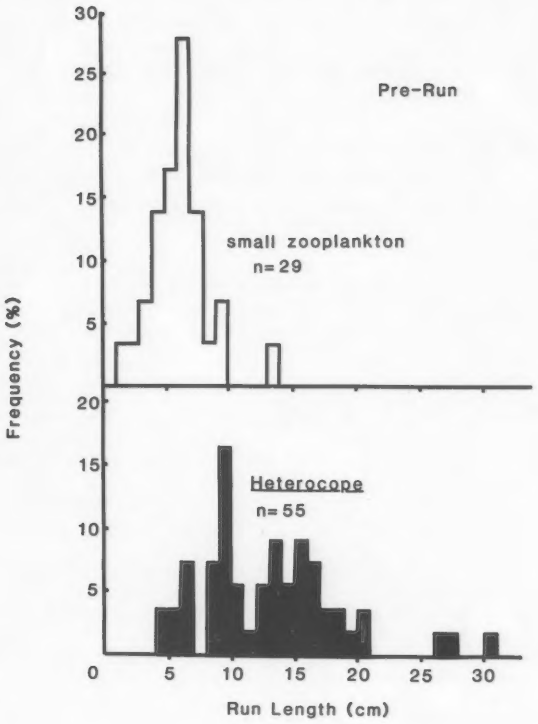
FIG. 3. Pursuit volumes. The upper panels indicate the distribution of pursuit angles for (a) large prey and (b) small prey in 10° increments. The lower panels illustrate the angle and distance of the pursuits on (c) large prey and (d) small prey. Measurements were made at 10°C.

swimming. Therefore, it appears that the grayling were instead searching during the pause. The run lengths were shortened, however, when the grayling fed on small versus large prey (K–S test, $p < 0.001$; Fig. 4). In fact, the average distance of the runs was 42 and 46% of the longest pursuit distance (maximum location distance) observed for large and small prey, respectively (Table 1), suggesting that the run length was related to maximum location distance (Vinyard and O'-Brien 1976).

Grayling also altered their run turn angles when they fed on small versus large prey (Fig. 5). When feeding on *Heterocope*, most run turns were between 30 and 100°, with some larger outliers; however, when feeding on small prey, over 90% of the run turns were less than 50° (Table 1). These distributions differed significantly (K–S test, $p < 0.001$). Run turns occur when no prey are detected. Comparison of the run turn angle with the observed pursuit angle (Fig. 3) illustrates that the run turn angles increase when location angles are larger. The observed variation in run length and run turn angle in response to different prey suggests that the grayling are varying the components of search, relative to their previous experience in detecting prey.

Discussion

Our results indicate that arctic grayling are saltatory searchers. Given that they search in this manner, why do they not stop as abruptly nor for as long as do the centrarchids? Why do they adjust their swimming speed in the presence of different-sized prey? We propose that, when feeding on small prey, slower swimming facilitates longer search pauses. Grayling,



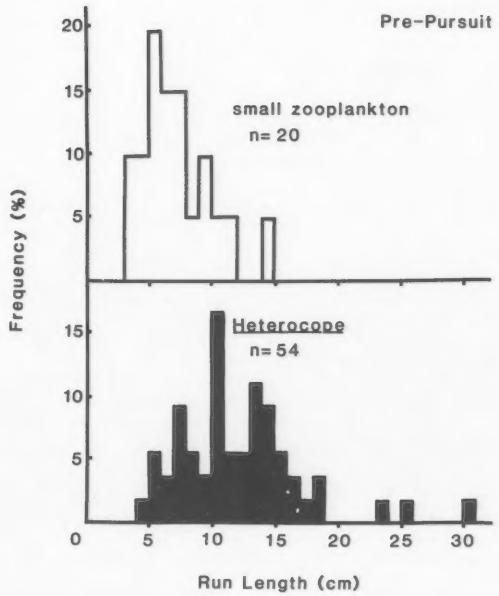


FIG. 4. Run length frequency. The distribution of run lengths prior to an unsuccessful pause (pre-run) are diagrammed for large and small prey (upper graph). The lower graph illustrates the run length frequency prior to a successful search (pre-pursuit). Measurements were made at 10°C.

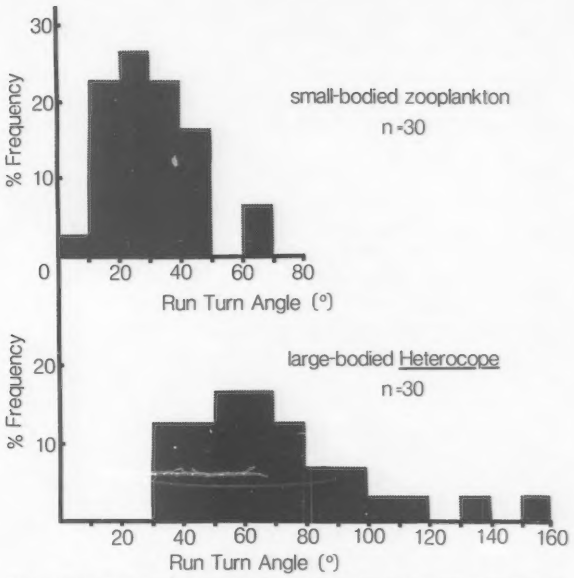


FIG. 5. Run turn angle distribution. The angles of reorientation during a turn (not followed by pursuit) are illustrated for small prey (top panel) and large prey (bottom panel). Measurements were made at 10°C.

TABLE 1. Means of run lengths, reaction distance, and turn angles for arctic grayling feeding on large (*Heterocope septentrionalis*) and small prey. RD = reaction distance; RL = run length.

	Search volume angle[a]	Max. RD	Mean RL	Ratio of RL/RD	% of new water[b]	Run turn angle[a]
Small prey	62°	14	6.5	0.46	68	25°
Large prey	80°	28	12.0	0.42	62	65°

[a]95% of the cases.
[b]Assuming a 90° search angle for both.

like other salmonids, are fusiform, with their pelvic fins placed well posterior to the pectoral fins. Such a body plan is well adapted for continuous swimming and allows salmonids to maintain position in flowing water (Alexander 1967; Webb 1984). However, such a fin placement does not allow for quick braking of forward motion, and attempts to brake quickly result in a positive pitching moment (Harris 1938; Gray 1968). In contrast, the close proximity of the pelvic and pectoral fins of centrarchids does provide a stable braking system. Thus, the fin placement of centrarchid fishes, such as the white crappie, facilitates braking, while that of the arctic grayling does not. To adjust search pause duration, grayling must regulate swimming speed. Increased swim speed erodes search efficiency in a cruise searcher; however, the adjustment of swim speed as a function of prey detectability is not sufficient evidence that a forager uses a cruise strategy.

Like the crappie (Evans and O'Brien 1986; Evans 1986), grayling appear to adjust the length of their repositioning runs on large versus small prey, such that the run lengths are approximately one half of the maximum location distance (reaction distance) to the prey being fed upon. If we assume a pie-wedge shaped search volume, with side equal to the reaction distance and angle equal to 90°, these runs would pro-

vide 62–68% new water for the next search (Table 1). Adjustment of the run length, as observed here, would not be expected of a cruise searcher. By adjusting the run lengths, a saltatory searcher can efficiently search for small prey without missing much water and without searching much area twice (W. J. O'Brien, B. I. Evans, and H. Browman, unpubl.). Because the entire search volume can be scanned at the beginning of the run, selective pressure may have resulted in the selection of planktivores which utilise a saltatory search behavior to increase their probability of prey detection.

Grayling also adjust their run turn angles in response to increased angles of prey location, a behavior which was also observed in the white crappie (Evans 1986). When the angle of the location volume is small, as was observed for grayling pursuing small prey, a forager need not turn far to see new water. As the angle of this location volume increases, larger run turn angles are necessary to avoid researching much of the same volume of water. A cruise searching forager would not benefit from the adjustment of run turn angle, observed in saltatory searchers, because of their continuous search behavior.

Cruise search may be a rare feeding strategy among visual planktivores due to the erosion of visual detection ability as a consequence of motion (Gendron and Staddon 1983); however, at low light or high turbidity, a cruise search behavior may be advantageous. Under such conditions, the maximum location distance is greatly reduced. Should the fish attempt to run one half of this distance, they would move very little between searches. As the maximum location distance is reduced further, the run lengths would also decrease until the fish, essentially, would not be able to reposition at all without swimming beyond the maximum location distance. We have preliminary evidence which indicates that white crappie do indeed switch to a cruise strategy under turbid conditions (B. I. Evans and W. J. O'Brien, unpubl.).

Instead of discrete ambush and cruise search strategies (Gerritsen and Strickler 1977), we have evidence for a gradient of behaviors, with saltatory search being an intermediate strategy in some predators' foraging repertoire. A saltatory searcher which makes shorter runs and briefer pauses becomes more like a cruise searcher. A saltatory searcher which makes longer runs and pauses becomes more like an ambush predator.

The tactic of searching during the pause does not rule out the possibility of detecting prey while moving, but suggests that the forager is most efficient by searching prior to the run. By confining search for prey to pauses, foragers may allow themselves the freedom to focus attention on their own predators during the run. Ground foraging, birds have been observed to raise their heads during a run (Pienkowski 1983), in apparent vigilence for predators. Possibly, planktivorous fish scan for predators at greater distances during the run, while searching at close range during the pauses. By utilising a saltatory search behavior, a forager may best be able to balance the conflicting demands of searching for food and avoiding predators, an option which would not be available to cruise searchers.

Virtually all fish are planktivores at some point in their life cycle, and the ability to feed effectively on zooplankton resources can determine the success of a species. Assuming that saltatory search is as common as it appears, this type of search behavior may have evolved independently in many groups, driven by the common constraints on visual foragers. During the arctic summer there are only two months when the zooplankton are abundant and the lakes clear of ice. To utilise these zooplankton resources, a planktivore must be capable of feeding efficiently. The observation that planktivorous arctic grayling, which cannot easily stop to search, also forage in a saltatory manner suggests that the advantages of a saltatory search strategy are greater than previously thought.

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Incubation of Eggs of Atlantic Salmon (*Salmo Salar*) from Different Norwegian Streams at Temperatures below 1°C

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Wallace, J. C., and T. G. Heggberget. 1988. Incubation of eggs of Atlantic salmon (*Salmo salar*) from different Norwegian streams at temperatures below 1°C. Can. J. Fish. Aquat. Sci. 45: 193–196.

Atlantic salmon (*Salmo salar*) eggs obtained from fish from five widely separated localities were incubated from fertilization until hatching at mean temperatures of 1.0 and 0.17°C. The results indicate that an existing model used to predict time of hatching in Atlantic salmon at temperatures greater than 2°C can also be applied for lower temperatures. The eggs hatched 250–260 d after fertilization at mean temperatures of 0.15–0.17°C (38–44 day-degrees) and after 206–221 d at temperatures of 0.97–1.03°C (205–214 day-degrees). There was no evidence of stock adaptation or of within stock adaptation to temperature at any of the different localities during the period from fertilization to hatching.

Des oeufs de saumon de l'Atlantique (*Salmo salar*), obtenus de poissons recueillis à cinq endroits éloignés l'un de l'autre, ont été incubés à des températures moyennes de 1,0 et 0,17°C du moment de la fertilisation jusqu'à éclosion. Les résultats révèlent qu'un modèle existant de prédiction du moment de l'éclosion des oeufs de saumon de l'Atlantique à des températures supérieures à 2°C peut aussi être appliqué à de plus faibles températures. Les oeufs ont éclos entre 250 et 260 j après la fertilisation quand la température moyenne était de 0,25 à 0,17°C (38–44 degrés-jours) et entre 206 et 221 j à une température de 0,97 à 1,03°C (205–214 degrés-jours). Il n'y avait aucune évidence d'une adaptation du stock ou d'une adaptation au sein du stock à la température aux différents endroits pendant la période entre la fertilisation et l'éclosion.

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The paucity of data on incubation of salmonid eggs at very low temperatures has been noted by several authors (e.g. Alderdice and Velsen 1978; Crisp 1981). Salmonids often spawn in the upper reaches of mountain streams, and their eggs experience temperatures approaching freezing for at least some of the incubation period. With Arctic populations, most of the incubation period must occur at extremely low temperatures. Predictions of incubation times for salmonid eggs at temperatures below 2°C are based on extrapolations from data gathered at higher temperatures. These estimates give predicted incubation periods for Atlantic salmon (*Salmo salar*) and trout eggs that range from 5.5 to about 9 mo (Crisp 1981). This experiment was carried out to obtain incubation data at the lower end of the temperature scale.

Another aspect of the study concerned comparison of populations. The anadromous habit, together with the accuracy with which Atlantic salmon return to their natal streams for spawning, makes it reasonable to assume that each salmon river contains its own salmon stock and that each stock may have become adapted to its own particular river (Behnke 1972; Thorpe and Mitchell 1981). Some such population specific adaptations have been demonstrated regarding morphology, spawning time, egg size, metabolic rate of developing eggs, pH tolerance, resistance to disease, and growth rate (Berg 1959; Gjedrem 1976; Andersen 1980; Kildal 1980; Riddell and Leggett 1981; Beacham and Murray 1985, 1986). Recent data on incubation of Atlantic salmon eggs from a northern Norwegian population showed that hatching occurred slightly later than predicted (Heggberget and Wallace 1984). The temperatures varied, however, during incubation, and no comparisons were made with other salmon populations. In the present study, eggs from five salmon stocks differing widely in geographical origin and environment were compared under almost identical experimental conditions.

Materials and Methods

Gametes were obtained by stripping ripe Atlantic salmon from five geographical sources: the Rivers Alta, Ims, Lone, and Sandvik as well as the research station for salmonids, Sunndalsøra (Fig. 1). The salmon from Sunndalsøra had been cultured through several generations, while the others were wild fish. In all cases the gametes were sent to the Institute of Fisheries, Tromsø, before fertilization was carried out. The gametes from the Alta salmon had been obtained from spawners captured in both the upper and the lower parts of the river. This permitted the establishment of several groups of fertilized eggs resulting from crosses of upper and lower river males with upper and lower females. As the Alta system might contain several, genetically separate salmon populations (Ståhl

TABLE 1. Incubation temperatures, observed times to 50% hatch, and heat sums to 50% hatch for the five egg stocks.

Egg stock	Fertilization date	Incubation temperature (°C)	Time to 50% hatch		Incubation temperature (°C)	Time to 50% hatch	
			Days	Degree-days		Days	Degree-days
Alta	Oct. 26	0.15±0.06	258	38	0.97±0.17	221	214
Ims	Dec. 13	0.17±0.08	251	42	1.00±0.22	206	205
Lone	Dec. 13	0.17±0.08	251	42	1.00±0.22	207	206
Sandvik	Dec. 13	0.17±0.08	260	43	1.00±0.23	209	209
Sunndalsøra	Dec. 22	0.16±0.08	267	44	1.03±0.28	207	214

FIG. 1. Locations from which the salmon eggs used in the experiment were obtained.

1981; Heggberget et al. 1986), a comparison of these combinations of gametes was of interest. The eggs were placed in the incubators at the experimental temperatures immediately after fertilization.

The equipment has been described previously (Sivertsen et al. 1983). Two incubation tanks were maintained at nearly constant experimental temperatures of 0.1 and 1.0°C. In each tank, duplicate 200-mL portions of each egg population were placed in separate compartments of perforated aluminum incubation trays. The eggs were observed daily and the time taken to 50% hatching was noted for each group.

Crisp (1981) analysed three models of the relationship between hatching and temperature. We have tested Crisp's models 1b and 3b:

(1a) $\log D = b \log (T - \alpha) + \log a$

$= -26562 \log (T - (-11.0)) + 5.1908$

TABLE 2. Observed (O) number of days (D) to 50% hatch at temperatures (T) of 0.15 – 0.17°C compared with predicted (P) values generated by equations 1b and 3b of Crisp (1981). The constants α, b, and b_1 are those calculated for Atlantic salmon.

	Alta (0.15°C)		Ims (0.17°C)		Lone (0.17°C)		Sandvik (0.17°C)		Sunndalsøra (0.16°C)	
	P	O	P	O	P	O	P	O	P	O
$\log D = b \log (T - \alpha) + \log a$	256	258	256	251	256	251	256	260	256	267
$\log D = \log a + bT + b_1T^2$	239	258	238	251	238	251	239	260	239	267

TABLE 3. Observed (O) number of days (D) to 50% hatch at temperatures (T) of 0.97–1.03°C compared with predicted (P) values generated by equations 1b and 3b of Crisp (1981). The constants α, b, and b_1 are those calculated for Atlantic salmon.

	Alta (0.97°C)		Ims (1.00°C)		Lone (1.00°C)		Sandvik (1.00°C)		Sunndalsøra (1.03°C)	
	P	O	P	O	P	O	P	O	P	O
$\log D = b \log (T - \alpha) + \log a$	213	221	211	206	211	207	211	209	210	207
$\log D = \log a + bT + b_1T^2$	202	221	203	206	203	207	202	209	201	207

(3b) $$\log D = \log a + bT + b_1 T^2$$
$$= -2.3921 - 0.0877T + 0.00166T^2$$

where D = incubation times (days), T = temperature (degrees Celsius), and a, b, b_1, and α are constants (Crisp 1981, table 2).

The temperatures from the present experiments were entered into the above equations and solved for D, which are compared with our observed time to 50% hatch.

Results

An initial egg mortality was noted in all egg groups. It could have been a result of one or several possible factors. Exposure to low temperature immediately after fertilization has been shown to cause mortality in eggs of Atlantic salmon (Peterson et al. 1977; Beacham and Murray 1985). Other possible causes of egg mortality are gamete transport, nonfertilization, and sensitivity to mechanical shock during egg manipulations. Mortality was about the same in all groups (10–20%), but no conclusion could be drawn based on differences in initial mortality. Times and heat sums (degree–days) to 50% hatching are shown in Table 1. No differences were found among the different Alta groups, so the Alta eggs are considered as one group. The small differences in average temperatures of incubation are due to the different fertilization dates and the slight temperature variations during incubation.

Tables 2 and 3 compare the present experimental data with values computed using Crisp's (1981) equations 1b and 3b, which were those found to provide the best fit to data obtained at higher temperatures. It can be seen that for temperatures of 0.15–0.17°C, our observed data correspond extremely well with those generated using Crisp's equation 1b. Equation 3b generates incubation times shorter than those observed.

The data obtained at about 1.0°C also fitted equation 1b well, and equation 3b was also fairly applicable at this temperature.

Discussion

Several factors may affect the development, time of hatching, and survival of fish eggs. Hamor and Garside (1976) found, for Atlantic salmon, that developmental rates to various structural stages of the embryos and hatching time were significantly correlated with temperature, rate of water exchange, and oxygen supply. In the present experiments, physical factors other than temperature were equalized for all groups of eggs.

Comparisons of models predicting rate of development of teleost eggs as a function of temperature suffer from a paucity of data at the lower end of the temperature scale (Alderdice and Velsen 1978; Crisp 1981). The data presented here were collected at experimental temperatures in this poorly defined region and are supplementary to the available information.

That incubation and hatching occur at all at the temperatures investigated here demonstrates that the Atlantic salmon is a cold-adapted species. A mean incubation temperature threshold of 1.7°C was shown to cause nearly total mortality in chinook salmon (*Oncorhynchus tshawytscha*) eggs (Combs and Burrows 1957), although it should be noted that no prefertilization temperature data were provided in that report. Threshold temperatures causing 50% mortality (fertilization to hatching) in North American Pacific salmon range from <1.6 to 3.8°C, that for chinook salmon being about 2.75°C (Velsen, Jensen, and Alderdice, Pacific Biological Station, Nanaimo, B.C., pers. comm.).

The use of heat sums (day-degrees) at very low temperatures is of doubtful value. As shown by Crisp (1981), a thermal sums equation may be obtained from his equation 1a. That is, if D (days) = aT^b and $b = -1.0$, then $D{\cdot}T = a$, a constant. Graphing the differences between observed and predicted ($D{\cdot}T = a$) incubation times at low temperatures shows an increasing divergence at temperatures below about 5°C, leading to the impossible result for temperatures of 0.15–0.17°C seen in Table 1.

In the present experiments with mean water temperatures, 50% hatch was observed 206–267 d after fertilization. In nature, incubation times will be shortened and modified by the rapid temperature increase during spring (Heggberget and Wallace 1984).

The relatively small differences in incubation times among the egg groups may also be of questionable significance. Temperature data were obtained for the Rivers Alta and Ims (Asvall 1982; Senstad 1980) and represent mean temperatures for the last 10-yr period. Alta is an extremely cold river in winter,

the temperature remaining stable near 0.1°C from about mid-November until the beginning of May. Ims, on the other hand, seldom falls below 1.0°C, and except during a short period from mid-January until mid-March, it is usually warmer than 2.0°C. The natural incubation time for salmon eggs from the Ims population, therefore, is much shorter than that experienced by Alta eggs. The fact that the Alta eggs took 7 d longer to hatch at 0.15°C and 15 d longer at 1.0°C than the Ims eggs may or may not be due to genetic influence. It could also be attributable to the very slight differences in average incubation temperatures.

No detailed temperature data were obtained for the other rivers. River Lone is expected to be similar to the Ims, while River Sandvik is relatively cold during winter. The Sunndalsøra salmon can be considered as an artificial population developed for the Norwegian fish farming industry.

From the examples given, it would appear that any genetic adaptation of salmon stocks to local conditions is not manifested in the relationship between temperature and incubation time. A similar result was obtained by Beacham and Murray (1986) in a study of Frazer River chum salmon (*Oncorhynchus keta*). They found no evidence for stock adaptation in the rates of development of eggs from fertilization to hatching.

We conclude that incubation times (fertilization to 50% hatch) for the five stocks of Atlantic salmon examined are well predicted by the equation $D = aT^b$ at temperatures below 1.0°C. Although development times predicted by thermal sums ($DT = a$) were reasonable at temperatures of about 1.0°C, they were not so at temperatures of about 0.15°C. We also conclude from the five stocks examined that genetic adaptation of stocks to local conditions is not shown in a possible relationship between temperature and incubation time.

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